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Effect of the NF-κB Pathway Agonist IL-1β in Lung Cancer Cells

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Zusammenfassung (Deutsch):

Die in der Mikroumgebung von Tumoren vorhandenen Entzündungsfaktoren sind für jeden Schritt der Tumorbildung und -entwicklung von entscheidender Bedeutung. Interleukin-1β (IL-1β) hat eine starke pro-inflammatorische Aktivität, die eng mit der malignen Transformation von Zellen, der Tumorbildung und der Metastasierung zusammenhängt. Canakinumab ist ein IL-1β hemmender Antikörper. Kürzlich zeigte eine große Studie mit Canakinumab bei Herzpatienten im Vergleich zu den Kontrollen eine geringere Inzidenz von Lungenkrebs bei Patienten, die mit Canakinumab behandelt wurden. Dieser Befund bildet die Grundlage für laufende klinische Studien mit Canakinumab bei Lungenkrebs.

Die Aktivierung des Nuklearfaktor κB (NF-κB)-Signalweges geht häufig mit Entzündungen oder Tumorwachstum einher. NF-κB ist nicht nur an der Zellzyklusregulation, Autophagie, Alterung, Apoptose, Entzündungsreaktionen, Immunreaktionen und anderen pathophysiologischen Prozessen beteiligt, sondern spielt auch eine wichtige Rolle bei der Entstehung, Entwicklung, Infiltration, Metastasierung und Behandlung von Tumoren.

Ziel dieser Arbeit war es, die Wechselwirkungen zwischen Lungenkrebszellen(H838, H1339, H1650 und H1975) und Immunzellen (Makrophagen), die IL-1β-Expression und die Wirksamkeit des NF-κB-Signalweg-Agonisten IL-1β gegenüber den Lungenkrebszelllinien H838 und H1975 zu untersuchen.

Die Lungenkrebszellen H838, H1339, H1650 und H1975 können die IL-1β-Sekretion durch Makrophagen fördern. In der Co-Kultur von Lungenkrebszellen und Makrophagen hat IL-1β eine höhere Expression als in Monokultur. Wir fanden keine IL-1β-Expression in einem Lungenkrebszell-Überstand in Monokultur. IL-1β wurde jedoch in einem Makrophagen-Überstand exprimiert. Dies bestätigt, dass das von uns nachgewiesene IL-1β von Makrophagen sezerniert wird.

Lungenkrebszellen fördern die IL-1β-Sekretion durch Makrophagen. In Co-Kultur

steht die Menge der IL-1β-Sekretion im Zusammenhang mit der Anzahl der Makrophagen: Die Anzahl der Makrophagen nimmt mit der Zeit ab, und die IL-1β-Sekretion nimmt entsprechend auch ab.

Interleukin-1β kann den NF-κB-Signalweg aktivieren und die Expression der Signalwegkomponenten sowohl in H838- als auch in H1975-Zellen beeinflussen. In H838-Zellen aktivierte IL-1β den NF-κB-Signalweg, und es wurde eine Herunterregulierung von IκBα, NFκB2 und RelA beobachtet. In H1975-Zellen aktivierte IL-1β den NF-κB-Signalweg, und es wurde eine Herunterregulierung von IκBα, IκBε, IKKβ, NFκB1, NFκB2 und RelA (pS529) beobachtet.

Lungenkrebszellen können Makrophagen zur Erhöhung der IL-1β-Sekretion stimulieren, was den NF-κB-Signalweg in diesen Zellen aktivieren und sie dazu veranlassen kann, ein für ihr Überleben günstigeres Wachstumsumfeld zu schaffen. Diese Wechselwirkungen sollten bei der Untersuchung und Modellierung der Expression von IL-1β als potentielles therapeutisches Ziel bei Lungenkrebs berücksichtigt werden.

Abstract (English):

The inflammatory factors present in tumour microenvironments are essential for each step of tumour formation and development. Interleukin-1β (IL-1β) has strong pro-inflammatory activity, which is closely related to the malignant transformation of cells, tumour formation and metastasis. Canakinumab is an IL-1β inhibitory antibody. Recently, a large trial of canakinumab in cardiac patients indicated lower lung cancer incidence in patients treated with canakinumab compared to the controls. This finding is the basis for ongoing clinical trials of canakinumab in lung cancer.

The activation of the nuclear factor κB (NF-κB) signalling pathway is often accompanied by inflammation or tumour growth. As well as participating in cell-cycle regulation, autophagy, aging, apoptosis, inflammatory responses, immune responses and other pathophysiological processes, NF-κB plays an important role in the occurrence, development, infiltration, metastasis and treatment of tumours.

This work aimed to investigate the interactions between lung cancer cells (H838, H1339, H1650 and H1975) and immunocytes (macrophages), IL-1β expression and the effectiveness of the NF-κB pathway agonist IL-1β against the lung cancer cell lines H838 and H1975.

The lung cancer cells H838, H1339, H1650 and H1975 can promote IL-1β secretion by macrophages. In the co-culture of lung cancer cells and macrophages, IL-1β has higher expression than in monoculture. We found no IL-1β expression in a monoculture lung cancer cell supernatant. However, IL-1β was expressed in a macrophage supernatant. This confirms that the IL-1β we detected is secreted by macrophages.

Lung cancer cells promote IL-1β secretion by macrophages. In co-culture, the amount of IL-1β secreted is related to the number of macrophages: the number of macrophages decreases with time, and IL-1β secretion decreases accordingly.

Interleukin-1β can activate the NF-κB pathway and affect the expression of the pathway components in both H838 and H1975 cells. In H838 cells, IL-1β activated the NF-κB pathway, and downregulation of IκBα, NFκB2 and RelA was observed. In H1975 cells, IL-1β activated the NF-κB pathway, and downregulation of IκBα, IκBε, IKKβ, NFκB1, NFκB2 and RelA (pS529) was observed.

Lung cancer cells can stimulate macrophages to increase IL-1β secretion, which can activate the NF-κB pathway in these cells and induce them to produce a more favourable growth environment for their survival. These interactions should be considered when studying and modeling the expression of IL-1β as a potential therapeutic target in lung cancer.

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NSCLC: non-small cell lung cancer

SCLC: small cell lung cancer

IL-1: interleukin-1

IL-1α: Interleukin 1Alpha

IL-1β: Interleukin 1 beta

IL-1R1: Interleukin 1 receptor, type I

IL-1R2: interleukin 1 receptor, type II

IL-1RL1: interleukin 1 receptor-like 1

IL-1RA: interleukin-1 receptor antagonist protein

NF-κB: nuclear factor kappa-light-chain-enhancer of activated B

TAMs: tumor-associated macrophages

DC: dendritic cells

MDSC: myeloid-derived suppressor cells

COX-2: cyclooxygenase 2

EMT: epithelial-mesenchymal transition

TWIST1: twist family b HLH transcription factor 1

SNAIL: zinc-finger transcription factor

ICE: IL-1β-converting enzyme

RHD: Rel homology domain

NLS: nuclear localization sequence

VEGF: vascular endothelial growth factor

FGF: fibroblast growth factor

MMP: matrix metalloproteinase

EGFR: epidermal growth factor receptor

CHART: continuous hyperfractionated accelerated radiotherapy

CARD: caspase activation and recruitment domain

PAMPs: pathogen-associated molecular patterns

TNFR: tumor necrosis factor receptor

ASC: Apoptosis-associated speck-like protein containing a CARD

RIP1: Receptor-Interacting Protein 1

TLR: toll-like receptor

STAT: signal transducer and activator of transcription

TCF: T-cell factor

CDK: cyclin dependent kinase

CKI: cyclin dependent kinase inhibitors

IκBα: nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha

IKK: IκB kinase

IRAK1: Interleukin-1 receptor-associated kinase 1

MYD88: Myeloid differentiation primary response 88

TNF: tumor necrosis factor，

TRAF2: TNF receptor-associated factor 2

TNF-α: tumor necrosis factor-α

LT-β: lymphotoxin-β

1. Introduction

1.1 Lung cancer and lung cancer therapy

1.1.1 Lung cancer

Lung cancer is the most commonly occurring cancer worldwide, and also the chief cause of cancer-related deaths. In lung cancer, malignant lung tumours are characterised by uncontrolled growth of cells in lung tissue [1]. This growth can spread beyond the lungs and into nearby tissues or other parts of the body. Among all the subtypes of lung cancer, non-small-cell lung cancer (NSCLC) and small-cell lung cancer (SCLC) are the most universal, these two subtypes account for about 80-85% of the incidence. The clinical manifestations of lung cancer are also diverse, mainly including respiratory symptoms, systemic symptoms and compression of adjacent tissues by the cancerous tissue [2]. Lung cancer has an insidious onset and early diagnosis is very important. In clinical practice, pathological examination, sputum cytology, X-ray chest film, chest computed tomography (CT) scans, serum tumour markers, molecular diagnosis and other diagnostic methods are commonly used.[3].Treatment options for lung cancer depend on the staging of the lung cancer and the progression of the tumour. In the oncological course of lung cancer, the common treatment options are surgery, radiotherapy and chemotherapy.

Lung cancer taken up 18.4% of all cancer-related deaths worldwide in 2018[4].In the USA, lung cancer ranks second in the age-adjusted morbidity rate of primary cancer types (after breast cancer)， and has the highest cancer-related mortality rate to date[5].Women's global age-standardised lung cancer incidence is lower than that of men in all regions; however, incidence in women is high in the regions of Northern and Western Europe, Oceania, and North America [4]. In 2017, almost half (47%) of the considered 222,500 new medical cases of lung cancer in the USA were female [5]. In the USA, NSCLC occupies 80–85% of all patients with lung cancer.

During the development of lung cancer, activation of oncogenes or inactivation of

tumour suppressor genes triggers the development of lung cancer. Mutations in these genes due to various causes trigger the development of cancer. As the level of genetic testing improves, more and more mutations associated with lung cancer are being identified. An example is the K-ras oncogene mutation, which is found in about 10-30% of all mutant subtypes of lung adenocarcinoma [6]. 4% patients in NSCLC patients are involved in EML4-ALK tyrosine kinase fusion genes. Epigenetic changes can also lead to the inactivation of tumour suppressor genes and cause the development of lung cancer. Common factors are changes in DNA methylation, modifications in histone tails or microRNA regulation [7]. During this process of tumour development, cancer cells become resistant to oxidative stress, which allows them to resist and exacerbate inflammatory diseases that suppress the activity of the immune system against the tumour [8]. The epidermal growth factor receptor (EGFR) contributes to tumour development by regulating cell proliferation, promotion of angiogenesis and tumour invasion. Its mutation and amplification are universial in NSCLC and therefore its mutation provides the basis for EGFR inhibitor therapy in lung cancer patients. As lung cancer genes are studied, more and more mutations or amplifications are being discovered. As lung cancer genes are studied, more and more mutations or amplifications of genes such as PIK3CA and BRAF are being discovered [6].

1.1.2 Classification of lung cancer

Lung cancer is divided into two categories, NSCLC and SCLC depending on the size of the tumour cells. The treatment and prognosis for the two are very different, and SCLC is generally a very malignant tumour.

Non-small-cell lung cancer

The three major subtypes of NSCLC are adenocarcinomas, squamous-cell carcinomas and large-cell carcinomas [9].

Lung adenocarcinomas are the first important subtype of NSCLC. These are divided into three types: acinar carcinomas, papillary adenocarcinomas and bronchioloalveolar

cell carcinomas. Generally, the age of onset is younger, and these are more common in women than in men. Most lung adenocarcinomas originate from small bronchial mucosal epithelial cells, while some originate from large bronchial tubes. Generally, the early symptoms are not obvious, and growth and development are slow; however, local invasion or blood metastasis can occur in the early stages, and these easily metastasise to the liver, brain, bone and other organs. They can also involve the pleura and cause pleural effusion [9].

Squamous-cell carcinomas include spindle-cell carcinomas (also known as squamous-cell). Squamous-cell carcinomas are the maximum frequent subtype of lung cancer, accounting for about half of all cases. They occur more often in men than in women, which is related to long-term heavy smoking. Squamous-cell carcinomas are derived from bronchial epithelial metaplasia; their degree of differentiation is different, their growth is slower and more progressive and the probability of metastasis is relatively small [9].

Large-cell carcinomas have a low clinical incidence. About half of cases originate in the large bronchi of the lungs. The lesions are more commonly seen as large peripheral masses. They are often accompanied by mediastinal lymphatic metastasis. They are more common in men than in women. Large-cell carcinomas have a high degree of malignancy and a low degree of differentiation. They are prone to brain metastasis and are associated with a poor prognosis [9].

Small-cell lung cancer

Among lung cancers, SCLC is the primary malignant, accounting for about one-fifth of primary lung cancer. It generally originates from the central bronchus, mostly as central lung cancer. It is more common in men than in women. Cancer cells are highly differentiated, fast growing and aggressive. Small-cell lung cancer is prone to long-distance metastasis, often metastasis to organs inculding the brain, liver, bone and adrenal glands. Clinical trial results show that undifferentiated small-cell carcinoma is more sensitive to radiotherapy and chemotherapy; however, radiochemotherapy is often

accompanied by serious toxicity, side effects and complications. Small-cell lung cancer generally has a poor prognosis [9].

Others

Adenosquamous carcinomas are rare in clinics and are mixed lung cancers comprising squamous-cell carcinomas and adenocarcinomas. They have no specific shape. Pulmonary neuroendocrine tumours are a unique subset of tumours. They share common morphology, ultrastructure, immunohistochemistry and molecular characteristics. Morphologically recognisable important types of neuroendocrine tumours which involve small-cell carcinomas, large-cell neuroendocrine carcinomas, typical carcinoid tumours and atypical carcinoid tumours.

1.1.3Lung cancer therapy

Treatment for lung cancer depends on the tumour staging, the extent of spread and the patient's physical condition. Current common treatments include palliative care, surgery, chemotherapy and radiotherapy. Targeted lung cancer treatment is becoming increasingly important for advanced lung cancer [10].

Surgery

Surgical removal of the tumour is the main treatment for patients with early stage NSCLC. Surgery can be very effective in removing the tumour and halting the progression of the disease [11]. For patients with SCLC, surgery is not the best option for treatment and it is not very effective for their treatment. After treating early SCLC with chemotherapy and radiotherapy, surgery may improve outcomes[12, 13].

Radiotherapy

Radiotherapy is usually performed concurrently with chemotherapy and can be used for patients with NSCLC that is unsuitable for surgery. Radiosurgery is a radiotherapy technique that performs precise large-dose computer-guided radiotherapy. For patients with lung cancer, radiotherapy is an effective treatment. Small doses of chest radiotherapy can be effective in controlling symptoms. High-dose palliative

radiotherapy has not been shown to prolong survival [14].

Chemotherapy

Chemotherapy is an effective treatment and adjuvant for lung cancer and is broadly used to treat patients. Especially in the treatment of SCLC patients and NSCLC patients who cannot tolerate surgery, chemotherapy acts a significant position in controlling the progress of the disease as one main treatment. Chemotherapy can be the mainstay of treatment for patients with advanced NSCLC and is effective in increasing their survival rates and improving quality of life.[15].

Targeted therapyand immunotherapy

With the study of lung cancer genes, targeted drugs are also emerging as a new option for lung cancer treatment. For patients with EGFR mutations, EGFR inhibitors can be an effective means of treatment, not only for halting the progression of the disease but also for improving the quality of life[16].

Immunotherapy has made rapid progress in NSCLC after years of exploration and clinical trials. Currently, immune detection site inhibitors, represented by anti-PD-1 and PD-L1 monoclonal antibodies, have been authorized as first and second line treatment in lung cancer clinics. Anti-PD-1 and PD-L1 monoclonal antibodies or combined immunotherapy comprehensively outperform traditional typical chemotherapy in the first-line treatment of NSCLC and will comprehensively and completely change the clinical treatment landscape for NSCLC patients [17].

1.2 IL-1β and IL-1β in lung cancer

1.2.1 IL-1β

In 1984, it was discovered that interleukin-1 comprises two different proteins, now known as interleukin-1α and interleukin-1β [18].IL-1β is also called leukocyte pyrogen, leukocyte endogenous mediator, monocyte cytokine and lymphocyte activation factor. It is a cytokine protein encoded by the IL1B gene in humans [19-22]. The precursor of

IL-1β is inactive and needs to be cleaved by cytoplasmic cysteine protease 1 (IL-1β convertase) to form mature IL-1β.

IL-1β is generated by macrophages in activate and is present in the proprotein form, which is hydro lysed and processed by caspase-1 protein to form its active form. The cytokine is a crucial mediator of inflammation and participates in various cellular activities, comparing cell proliferation, differentiation and apoptosis. It was found that this cytokine induces cyclooxygenase-2 in the central nervous system, it contributes to the hypersensitivity of inflammatory pain [23].

IL-1β combined with IL-23 can induce γδT cells to express IL-17, IL-21 and IL-22. This expression and induction occur in the absence of other signals. This indicates that IL-1β is incorporated in the regulation of autoimmune inflammation [24].

The inflammatory body recognizes a danger signal and activates the pro-inflammatory process and the production of inflammatory factors (IL-1β and IL-18). The pyran domain, nucleotide-binding domain and leucine-rich repeat sequence are the three constituent domains of the NOD-like receptor family pyrin domain-containing 3 (NLRP3). The development of a number of diseases has been shown to be associated with NLRP3 activation, not only in relation to inflammatory issues, but also in metabolic, cardiovascular and neurological disorders, including: atherosclerosis, type 2 diabetes (T2D), obesity, and neurologic disorders [25-27].

Processing of IL-1 agonistic molecules

Interleukin-1α and IL-1β are both structured as 31-kD precursors belonging to IL-1 family, which also known as pro-IL-1. They can be further reduced their seize to 17-kD by protease. This kind of carboxyl terminal peptides are the mature forms of IL-1α and IL-1β [28]. IL-1β-converting enzymes is a cysteine protease (ICE), also known as caspase-1. It is activated in the cytoplasm of the inflammatory process and then cleaves the pro-IL-1β into its mature secreted 17-kDC-terminalform (IL-1β) [29-36]. Caspases are strictly specific, generally recognizing the tetrapeptide sequence and cleaving the C-terminal peptide bond to the aspartate residue, this is also the structural basis for catalytic IL-1β [37]. Caspase-1 is normally expressed as a non-activate zymogen that

undergoes dimerization and activated by the inflammasomes (large multi-protein complexes) [38-42]. The formation of inflammasomes is caused by various microbe. Their products or stress-related signals will trigger and activate acute inflammation and cause the secretion of caspase-1. Additionally, caspase-1 induces cell death in the form of pyrophosphorylation, which can limit the spread of pathogens within macrophages[43-47]. The process of pyrophosphorylation also induces the generation of IL-1β and IL-18, leading to the immune cells recruitment and the inactivation of released pathogens [48].

To date, the most studied are inflammasomes containing NOD-like receptors (NLR) sensors, including NLRP1 - 4[43]. Each kinds of sensor protein has its specific ligand, which has been widely characterized. For the assembly and activation of inflammatory body, each component involved in the process must be present in the identical cell. Caspase-1 and apoptosis-associated speck-like protein (ASC) are generally expressed in cells and tissues, even though the expression of inflammatory corpuscle sensors is limited, indicating that cell or tissue type-specific mechanisms can perceive tissue disturbance. It is increasingly recognized that different cell types of inflammatory bodies may elicit different but often complementary responses.

1.2.2 IL-1β in lung cancer

The central function of the tumour microenvironment in the occurrence and development of tumours has been widely recognised by the biomedical community; anyhow, the exact mechanism of action remains unclear. During tumour development, infiltration of inflammatory cells like macrophages often occurs, which is also an important feature of tumours [49]. Tumour-associated macrophages (TAMs) are macrophages that infiltrate the tumour tissue, and their number is closely related to the poor prognosis of the tumour [50]. Activated TAMs infiltrate the tumour microenvironment and produce cytokines and chemokines through autocrine and paracrine methods to regulate tumour growth [51]. Many macrophages infiltrate human

lung adenocarcinomas as well as squamous-cell carcinomas, while normal lung tissue contains only a few macrophages [49]. Macrophages can secrete IL-1β, tumour necrosis factor and other cytokines, which play a tremendous role in tumour proliferation and development.

IL-1βis an important pro-inflammatory factor expressed by macrophages. It can promote the development of lung cancer, colorectal cancer and other tumours [52-54]. Its mechanism includes promotion of tumour cell growth by activating the NF-κB signalling pathway [55]. Interleukin-1βpromotes angiogenesis and can increase the formation of blood vessels around lung tumours [55]. It promotes the development of lung cancer by inhibiting the microRNA-101/Lin28B pathway of lung cancer cells [54]. Moreover, IL-1β induces GSK3β phosphorylation, stabilises β-catenin and enhances TCF-dependent gene activity, thereby activating the tumour cell Wnt/β-catenin signalling pathway to promote tumour proliferation[53].

The stimulation of IL-1β significantly reduced the expression of Beclin1 protein in lung cancer cells. Beclin1 is a tumour suppressor protein, a specific gene for mammals that participates in and stimulates autophagy [56]. IL-1β is likely to inhibit the expression of Beclin1, thereby inhibiting autophagy of lung cancer cells, resulting in enhanced lung cancer cell proliferation.

1.3 IL-1 Receptor 1

Interleukin 1 receptor 1, type I (IL-1R1), also acknowledged as cluster of differentiation 121a (CD121a), is one of the main interleukin receptors. It has high binding affinity with IL-1β. IL-1R1 also denotes its human gene.

IL-1R1 encodes the cytokine receptor belonging to the interleukin-1 receptor superfamily. The superfamily can be divided into two main subfamilies, IL-1R1 belongs to one of the sub family which has three immunoglobulin domains extracellularly. IL-1R1 is a ligand-recognition receptor [57]. It exerts its function by

acting as a receptor for IL-1α, IL-1β and interleukin-1 receptor antagonist protein (IL-1RA). In addition, theIL-1β–IL-1RI–IL-1RA complex is a critical signaling-competent [58]. Of note, it is a significant mediator appeared in immune responses and inflammation induced by many cytokines. The gene forms interleukin-1 receptor, type II (IL-1R2), interleukin-1 receptor-like 2 (IL-1RL2) and interleukin-1 receptor-like 1 (IL-1RL1) to build cytokine receptors in the localised area gene cluster, chromosome 2q12[59, 60]. Binding to agonists contributes to activation of NF-κB signaling pathway. Simultaneously, active NF-κB prompts assorted pro-inflammatory genes expression, inclusive of those encoding IL-1β.

1.4 NF-κB and NF-κB signalling pathway

1.4.1 NF-κB

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB） is a famous well-known intracellular nuclear transcription factor. It is broadly distributed in diversity cell types of animal. NF-κB is involved in many of the body's reactive processes, inflammatory responses, stress responses, but over-activation can also cause many diseases, commonly rheumatoid arthritis and heart disease [61-64].

Normally, NF-κB binds to inhibitory proteins to compose inactive complexes in the cytoplasm. NF-κB can be activated only when a factor such as tumour necrosis factor is used with the corresponding receptor. However, in the case of viral infection, reactive oxygen intermediates, etc., NF-κB could be activated directly. Activation occurs by phosphorylation of the inhibitory protein, which changes its conformation so that the complex can be easily separated and NF-κB is detached from the complex to form activated NF-κB. NF -κB can connect with DNA and initiate or repress the transcription process of the relevant genes [65-67].

1.4.2 NF-κB family

Members of the NF-κB family are categorized as NF-κB/ Rel proteins because of their structural homology with the retroviral oncoprotein v-Rel. NF-κB1 (p50), NF-κB2 (p52), RelA (p65), RelB and c-Rel are the five members of the NF-kB family. p65/p50 subunit-forming NF-KB1 dimeric proteins and RelB/p52 subunit-forming NF-kB2 dimeric proteins are the two most common groups of proteins, so NF-KB proteins are also classified as these two proteins. NF-κB1 and NF-κB2 are produced by cleavage of the pII0 and p105 precursors, respectively. NF-κB1 and NF-κB2 generally form dimers with other representatives of the NF-kB family and remain in the cytoplasm. RelA, RelB and c-Rel proteins are precursor-less. NF-κB can bind to inhibitory proteins that inhibit NF-κB activity, preventing NF-κB from forming dimers and entering the cytosol. And in the cytoplasm NF-κB can only exist as a complex formation. When stimulated by tumour necrosis factor or inflammatory factors, NF-κB inducible mitogen kinase can be activated, which in turn phosphorylates IκBa or IκBβ. Phosphorylated IkBa or IkBβ can be degraded, allowing NF-κB activation [61].

1.4.3 NF-κB signalling pathway

NF-κB proteins are nuclear transcription factors in the signalling pathway. Normally, NF-κB binds to repressor proteins and is present in the cytoplasm in an inactive complex. Only after activation and separation from the repressor protein can they gain biological activity and enter the nucleus to function. There are three main signalling pathways that mobilize NF-kB, comprising the canonical pathway, the non-canonical pathway and the bypass pathway. nF-kB1, RelA and c-Rel are all activated via the canonical pathway. While, nF-kB2 as well as RelB are activated via the non-canonical pathway [68].

The canonical pathway begins with pro-inflammatory cytokines and pathogen-associated molecular patterns (PAMP) of the cell surface receptor. Once binding to the ligand molecule, the receptor conformation will be altered and then activates the IκB kinase (IKK) complex. The most universal forms of the complex are heterodimers of

the catalytic subunits IKKα and IKKβ as well as the IKKγ regulatory subunit. The IKK complex in activate acts mainly via IKKβ in an IKKγ-dependent way, catalyzing the phosphorylation of IκB, polyubiquitination (corresponding to the Ser32 and Ser36 sites) and final recognition and degradation by the 26S proteasome. The released active NF-κB dimer enters the nucleus and binds to DNA, which then activates downstream gene transcription processes [68].

The non-canonical pathways include transfer of signals into the cytoplasm via LT-β or BAFF receptors. NF-κB-inducible kinase (NIK) is phosphorylated and subsequently phosphorylates the IKKα homodimer. The target of IKKα is NF-κB2/ p100. Nevertheless, the phosphorylation-dependent ubiquitination of p100 leads to degradation of only one half of corresponding inhibitory C-terminus. When the C-terminal part is degraded, the N-terminal portion of NF-κB will be released. Because the RHD of p100 is normally related to RelB, activation of the 'optional' pathway leads to a nuclear transfer of the p52-RelB dimer. The dimer eventually binds to DNA sequences and activates downstream gene transcription [68, 69].

Ubiquitin acts a very large function in the regulation of this pathway. Ubiquitin is a kind of protein with a small size which can be found in most eukaryotic cells. Its main function is to label proteins that need to be broken down to hydrolyze them. Ubiquitination is an enzymatic process of post-translational modification (PTM) of proteins. In this process, the carboxyl group of the terminal glycine from the di-glycine module of activated ubiquitin is linked to the lysine epsilon amino group in the modified protein to form an amide bond. When cells are not stimulated, NF-κB and NF-κB inhibitors will be simultaneously present in cytoplasm as a complex. When cells are stimulated by inflammatory factors, for example, the IKK complex will phosphorylate IKKB, followed by ubiquitination and degradation of phosphorylated IκB by the 26S proteasome. Activated NF-κB can enter the nucleus then participate in the modulation of the expression of many genes. Ubiquitination acts a major process in the overall regulation of NF-κB pathway[70, 71].

1.4.4 NF-κB signalling pathway with lung cancer

Lung cancer is mainly caused by environmental factors, especially closely related to smoking and asbestos exposure, and is also associated with chronic inflammation and NF-κB activation. NF-κB activation is a prominent stepin EGFR mutant lung cancer, where suppression of the EGFR oncogene rapidly activates the NF-κB pathway and promotes tumour formation via IL-6 induction [72].

In lung cancer cell lines A549 and H1299, activation of the NF-κB signaling pathway upregulated the activity and proliferation-related proteins expression and contributed to the proliferation of tumor cells [73]. Activation of the NF-κB pathway promoted anti-apoptosis in tumour cells, enhanced resistance to chemotherapeutic agents and promoted tumour genesis and progression. In the A549 cell line, Bin1 was used to restrain the phosphorylation of NF-κB, which inhibited the growth of tumour cells by suppressing the NF-κB signalling pathway. The expression of N-cad and MPP-9 levels decreased after pathway inhibition, thus inhibiting tumour cell metastasis and proliferation.NF-κB signalling pathway can also promote tumour angiogenesis and thus tumour growth. activation of NF-κB signalling pathway promoted increased expression of integrinβ1, MMP2, MMP9 and other proteins and was also associated with upregulation of TWIST1, ZEB2 and SNAIL2[74, 75]. The protein IKKβ also plays a major role in this signalling pathway. In the signalling pathway, lung cancer cell proliferation was found to be significantly reduced following IKKβ deletion. This also suggests that IKK-mediated phosphorylation of IκB was shown to be largely dependent on the IKKβ catalytic subunit in the IKK complex [76].

With the increasing number of research findings on NF-κB signalling pathway, we have learned more about its role in tumours. In-depth research can lead to novel targeted drugs based on their different targets in tumours, which in combination with minimally invasive surgery and adjuvant radiotherapy will significantly extend the survival of tumour patients. It is believed that as the research on tumours progresses, more NF-κB signalling pathways will be discovered in tumours again, contributing to the improvement of survival rates of tumour patients.

2 Methods and Materials

2.1 Biological material

Tumor cell lines

- H838: ATCC no. CRL 5844, human non-small cell lung carcinoma (Biochem, 1996).
- H1339: DSMZ no. ACC 506, human small cell lung carcinoma (Phelps et. al., 1996).
- H1650: ATCC no. CRL 5883, human non-small cell lung carcinoma (Sordella et. al., 2004).
- H1975: ATCC no. CRL 5908, human non-small cell lung carcinoma (Sordella et. al., 2004).
- Macrophage: ATCC no. CRL 9850, monocyte/macrophage (Collins et. al., 1995).

2.2 Lab material and equipments

2.2.1 Laboratory consumables

- Culture flasks: 75 cm^2, Cat. 658175, Greiner Bio-One GmbH, Frickenhausen, Germany.
- Cryotubes: Cryo Vials, Cat. 121 277, Greiner Bio-One GmbH, Frickenhausen, Germany.
- Pipettes: 10 ml, Cat. 47110, Sterilin Ltd. Caerphilly, UK; 25 ml, Cat. 4251, Corning Incorporated, US.
- Centrifuge tubes: 50 ml, Cat. 227 261, Sarstedt AG & Co., Nümbrecht, Germany.
- Multi-well dishes: 96-well, Cat. 353072, Falcon, BD Biosciences Labware, NJ, US.

2.2.2 Lab equipments

- Centrifuge: Hettich EBA 12R and Universal 16A with eightfold rotor, Minnesota, US.
- Electronic pipette controller: Eppendorf-Netheler-Hinz GmbH, volume range 20-

200 μl, Hamburg, Germany.

- Eppendorf pipettes: volume range (0.5-10 μl, 10-100 μl, 20-200 μl, 100-1000 μl), Mettler toledo, Zurich, Switzerland.

2.3 Cell culture

2.3.1 Cell culture medium and supplement

- RPMI 1640: Cat. F1415, Biochrom AG, Berlin, Germany.
- Penstrep: penicillin (conc. 10,000 IU/ml) and streptomycin (conc.10,000 IU/ml), Cat. P11-010, PAA Laboratories GmbH, Pasching, Austria.
- Fungizone: amphothericin B, 250 μg/ml, Cat. P11-001, PAA Laboratories GmbH, Pasching, Austria.
- L-glutamine: 200 mM, Cat. M11-004, PAA Laboratories cell Culture Prodects, Pasching, Austria.
- Trypsin-EDTA: 0.05%/0.02% (dissolved in D-PBS), Cat. L11-004, PAA Laboratories cell Culture Prodects, Pasching, Austria.
- FBS: REF. 16170-078, Gibco, Darmstadt, Germany.
- PBS: Phosphate buffered saline without calcium and magnesium, Cat. H15-002, PAA Laboratories cell Culture Prodects, Pasching, Austria.
- Ethanol: 100%, Pharmacy, Klinikum Großhadern, Munich, Germany.
- DMSO: dimethyl sulfoxide, 5 ×10 ml. Cat. D2650, Sigma-Aldrich, St. Louis, US.

2.3.2 Cell culture equipments

- Laminar airflow: Heraeus, Munich, Germany.
- Incubator: Heraeus, Munich, Germany.

2.3.3 Cell culture medium recipe

FBS, L-glutamine Penstrep and Fungizone were added to RPMI 1640 medium to create the working medium we needed. Make the concentration of FBS 10% by volume, L-

glutamine at 200 mol/l, Penstrep at 10,000 IU/ml and Fungizone at 2.5 μg/ml.

2.3.4 Mono-culture of lung cancer cell lines and macrophages

H838, H1339, H1650, H1975 and macrophage were each placed in medium culture flasks containing 15 ml of medium and cultured in the incubator containing of the temperature is 37 and the CO_2 concentration is 5%. During cell culture, the solution needs to be changed once every two days, depending on the growth of the cells. When the cells have grown to about 75% of the total area of the culture flask, the cells need to be split with trypsin-EDTA solution. The collected cells are placed in lyophilised tubes containing 10% DMSO medium and stored frozen in liquid nitrogen for future use.

2.3.5 Lung cancer cell lines with Macrophages co-culture system

Lung cancer cell lines (H838, H1339, H1650 and H1975) and macrophages were co-cultured respectively in a ratio of 5:1 under the incubator containing of the temperature is 37 and the CO_2concentration is 5% for 7 days. Cell culture supernatants were collected at predetermined time points.

2.3.6 Lung cancer cell lines treated with IL-1β system

H838 and H1975 cells were incubated in medium culture flasks containing 15 ml of medium and cultured in the incubator containing of the temperature is 37 and the CO_2concentration is 5%. Afterward treated with adding IL-1β (the concentration of 2 pg/ml) medium for another 24h.

2.4IL-1β and IL-1R1 ELISA

2.4.1 ELISA Material and equipment

- Human IL-1β/IL-1F2 Immunoassay: Cat. HSLB00D, R & D Systems, Inc, Minneapolis, US.
- Human IL-1β HS Microplate: Cat. 898340, R & D Systems, Inc, Minneapolis, US.
- Human IL-1β HS Standard: Cat. 898437, R & D Systems, Inc, Minneapolis, US.
- Human IL-1β HS Conjugate: Cat. 898437, R & D Systems, Inc, Minneapolis, US.
- Assay Diluent RD1-63: Cat. 895352, R & D Systems, Inc, Minneapolis, US.
- Calibrator Diluent RD5T: Cat. 895175, R &D Systems, Inc, Minneapolis, US.
- Wash Buffer Concentrate: Cat. 895003, R & D Systems, Inc, Minneapolis, US.
- Stop Solution: Cat. 895032, R & D Systems, Inc, Minneapolis, US.
- Color Reagent A: Cat. 895000, R & D Systems, Inc, Minneapolis, US.
- Color Reagent B: Cat. 895001, R & D Systems, Inc, Minneapolis, US.
- Streptavidin Polymer-HRP Diluent: Cat. 898387, R & D Systems, Inc, Minneapolis, US.
- Streptavidin Polymer-HRP(100X): Cat. 898350, R & D Systems, Inc, Minneapolis, US.
- Human IL-1R1 Elisa kit: Cat. DY269, R & D Systems, Inc, Minneapolis, US.
- 96 well microplates: Cat. DY990, R & D Systems, Inc, Minneapolis, US.
- Plate sealers: Cat. DY992, R & D Systems, Inc, Minneapolis, US.
- PBS: 0.2μm filtered, Cat. DY006, R & D Systems, Inc, Minneapolis, US.
- Wash Buffer: Cat. WA126, R & D Systems, Inc, Minneapolis, US.
- Reagent Diluent: 0.1% BSA in PBS, Cat. DY995, R & D Systems, Inc, Minneapolis, US.
- Human IL-1R1 Capture Antibody: 720μg Cat. 840560, R & D Systems, Inc, Minneapolis, US.
- Human IL-1R1 Detection Antibody: 3.6μg Cat. 840561, R & D Systems, Inc, Minneapolis, US.
- Human IL-1R1 Standard: 110ng Cat. 840562, R & D Systems, Inc, Minneapolis,

US.

- Streptavidin-HRP: Cat. 840563, R & D Systems, Inc, Minneapolis, US.
- Berthold Technologies: Cat. LB940, Berthold, Germany.

2.4.2 Collect cell culture supernatants

H838, H1339, H1650, H1975 and macrophage cells were cultured in medium culture flasks. Cell culture supernatants were collected at 24h. Lung cancer cells and macrophages were then co-cultured respectively in a ratio of 5:1 under theincubatorof the temperature is 37 and the CO_2concentration is 5% for 7 days. Cell culture supernatants were collected at every 24h.

2.4.3 Detect IL-1β expression in supernatants

Human IL-1β HS standard

Reconstitute the Human IL-1β HS standard with distilled water. The standard solution must be well stirred and mixed and left to stand for at least 15 minutes before it is prepared. Using 8 tube to produce a dilution series. Pipette 100 µl 80 pg/ml standard into the first tube. Pipette 900 µl of Calibrator Dilution RD5T into the first tube to give a total volume of 1000 µl of liquid in the first tube and mix well. Then pipette 500 µl from the previous tube to the next tube in sequence. After the above procedure, we will have a dilution series of 8 pg/ml, 4 pg/ml, 2 pg/ml 1 pg/ml 0.5 pg/ml 0.25 pg/ml and 0.125 pg/ml. During this procedure, whenever the liquid is to be transferred once, the liquid in the current tube should be well shaken and mixed. The 8.0 pg/ml standard in this dilution series is the high standard in the series. In this series, the calibration fluid dilution RD5T will be used as a zero standard as a control (0 pg/ml).(Figure 1)

Figure 1

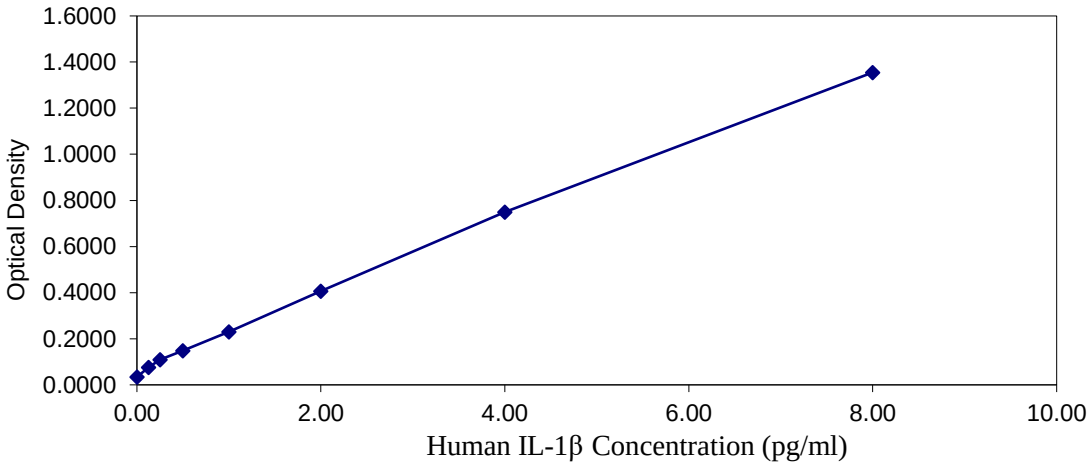

Figure 1. ELISA standard curve of Human IL-1β

Reagent preparation

1. Wash Buffer: First observe the state of the concentrate to check if crystals have formed, if they have, they need to be placed in a warm environment to promote their dissolution until they disappear. Dilute the concentrate to 1000 ml to obtain the required concentration for the experiment.

2. Substrate Solution: Colour reagents A and B should not be configured too early to prevent failure. It is usually sufficient to mix them within 15 minutes prior to use and must be stored away from light. 200 μl of the mixture should be added to each well at the time of use.

3. Streptavidin Polymer-HRP (1X): To configure the 1X Streptavidin Polymer-HRP add 0.215 ml of Antibiotic Streptavidin Polymer-HRP (100X) directly to the Antibiotic Streptavidin Polymer-HRP diluent. Allow to mix well and it is ready for use.

Assay procedure

1. Take out the microplate strips from the foil pouch containing.

2. Add 50μl of Assay Diluent RD1-63 per well.

3. Add 100 μl of standard, experimental samples to each well and cover with the tape provided. Place the prepared 96-well plate on a horizontal orbital microplate shaker (parameter: 0.12" orbit, 500 rpm) and incubate for 2 hours at room temperature.

4. Remove the liquid from each well and add 400 μl of wash buffer per well for washing. This process needs to be repeated 2 times, for 3 times washes totally. The third rinse is done by draining the rinse solution thoroughly. Turn the 96-well plate upside down and blot up the liquid with a clean paper towel.

5. Add 200 μl of Human IL-1βHS conjugate per well of a 96-well plate and cover with fresh tape. Place the 96-well plate on an oscillator and incubate for 1 hour at room temperature.

6. Wash the 96-well plate clean as in step 4.

7. Add 200 μl of Streptavidin polymer HRP (1X) to every well of a 96-well plate and cover with fresh tape. Place the 96-well plate on an oscillator and incubate for 30 minutes at room temperature.

8. Wash the 96-well plate clean as in step 4.

9. Add 200 μl of Substrate Solution to every well of a 96-well plate and cover with fresh tape. Place the 96-well plate on an oscillator and incubate for 30 minutes at room temperature. This step needs to be carried out in a light-proof environment.

10. Add 50 μl of Stop Solution per well and leave to observe at room temperature. The color in the observation wells should shift from blue to yellow. If the color changed not obviously, shake lightly to make full contact.

11. The absorbance of each well at 450 nm was measured using the zymograph. This process should be completed within 30 minutes; leaving it for longer will result in inaccurate readings.

2.4.4 Detect IL-1R1expressionin lysates

Cell lysates

Wash the cells with PBS and remove all residual PBS to ensure no residue remains before adding lysis solution. Keep the concentration of cells in the lysis buffer to around 1 x 10^7 cells/ ml. Blast the cells with a pipette and allow them to shake evenly in the lysis buffer for 30 minutes at 2-8°C. This will help the cells to lyse adequately. The lysed solution is centrifuged at a speed of 14,000 rpm for 5 minutes in a centrifuge at 4°C. After centrifugation, the supernatant need to be transferred to a new tube. The cell lysate should immediately be used or dispensed and stored at low temperature normally ≤ -70° C.

Recombinant human IL-1R1 standard

1Recombinant Human IL-1R1 standard with distilled water. Stir the standards until well mixed and allow to keep at room temperature for one quarter before diluting for use. Using 8 tube to produce a dilution series. Transfer 500 µl of the standard solution into the first tube with pipette. Then add 500 µl of the reagent dilution into the first tube to bring the first tube to 1000 µl. Pipette 500 µl from the first tube into the following tubes in sequence. After these operations, we obtain dilution series which are with standard concentrations of 8×10^3pg/ml, 4×10^3pg/ml, 2×10^3pg/ml, 1×10^3pg/ml, 0.5×10^3pg/ml, 0.25×10^3pg/ml and 0.125×10^3pg/ml in that order. Each time the liquid in each tube is thoroughly mixed before transferring to the next tube. The 8000 pg/ml standard in the first tube is the “high standard”. Use the reagent diluent as a “baseline zero standard” (0 pg / ml). (Figure 2)

Figure 2

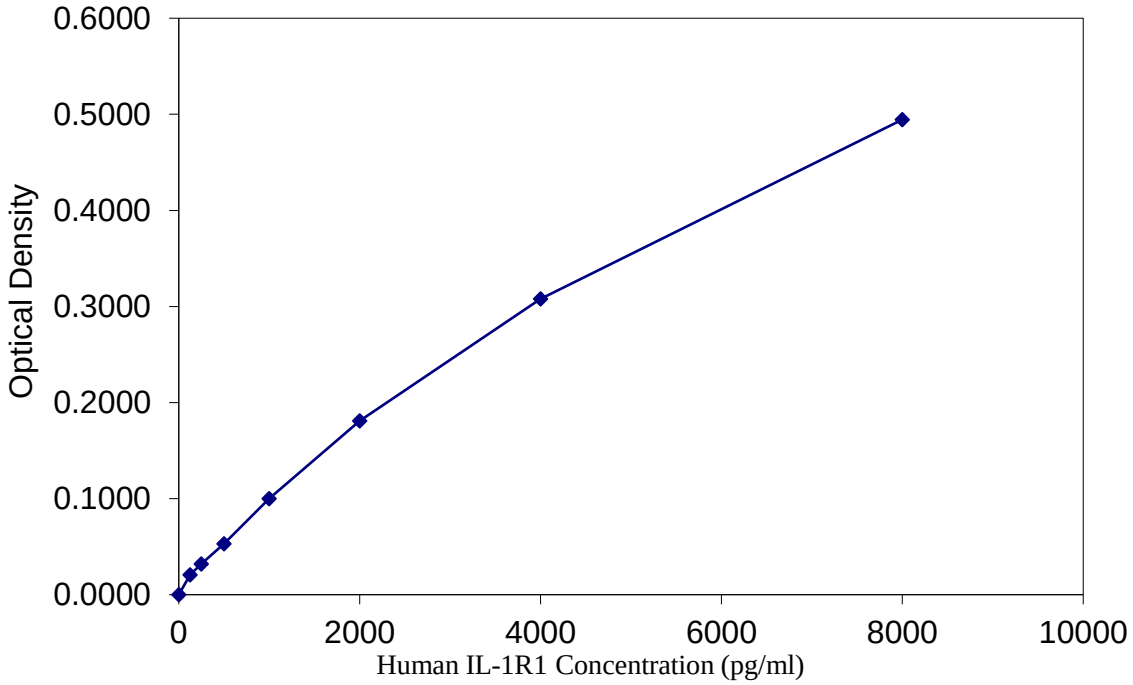

Figure 2. ELISA standard curve of Human IL-1R1

Reagent preparation

1. Streptavidin-HRP: streptavidin 1.0 mL conjugated to horseradish peroxidase. Use Reagent Diluent to dilute to the working concentration particular on the vial label.

2. Mouse anti-Human IL-1 RI Capture Antibody: This step requires dissolution to 1.0 ml PBS solution. Dilute the PBS solution without carrier protein to the desired experimental concentration of 4 μg/ml.

3. Biotinylated Goat anti-Human IL-1 RI Detection Antibody: This step requires dissolution to 1.0 ml Reagent Diluent solution. Dilute the Reagent Diluent solution without carrier protein to the desired experimental concentration of 20μg/ml.

Plate preparation

1. Dilute the capture antibody with PBS solution to the working concentration of 4 μg/ml as required for the experiment and mix well. Add the diluted capture antibody into a 96-well microtiter plate 100 μl per well. Keep the sealed 96-well plate at room temperature overnight.

2. Remove the liquid from the air and wash with washing buffer, adding 400 μl of washing buffer each time to wash, this step is repeated 3 times. After the last wash, the excess wash solution is blotted up with a clean paper towel so as not to interfere with the next step.

3.Add 300 μl of Reagent Diluent per well and incubate for 1 hour.

4.The 96 well plate is washed another 3 more times following step 2. Once this step has been completed, the 96-well plate is ready for the later operation of adding samples.

Assay procedure

1. 100 μl of sample or standard solution was added to each single well and seal the well with a new fresh adhesive strip, incubate for 2 hours at room temperature.

2. After removing the liquid, wash with Wash Buffer. Add 400 μl of Wash Buffer per well for rinsing, with each rinse being sufficient to prevent any residue. The process is repeated two times for altogether 3 rinses. After the third rinse, blot dry with a clean paper towel to prevent any residue of rinse solution from affecting the following operations.

3. Add 100 μl of the 20ng/ml Detection Antibody per well. Cover plate with a new fresh strip and incubate 2 hours.

4. Follow the second step to rinse the 96-well plate.

5. Add 100 μl of Streptavidin-HRP working dilution per well. Cover the plate and

incubate at room temperature for 20 minutes. The procedure needs to be protected from light to prevent the reagents from being affected by light and the experiment not working well.

6. Follow the second step to rinse the 96-well plate.

7. Add 100 µl of Substrate Solution to each single well. Incubate for 20 minutes at room temperature. The procedure needs to be protected from light to prevent the reagents from being affected by light and the experiment not working well.

8. Add 50 µl of Stop Solution to each single well. This process can be done with a slight shaking to promote adequate mixing of the solution for best results.

9. Determine the best density of every well quickly, with a microplate reader (parameter mode: 450 nm).

2.5 Human NF-κB pathway array

2.5.1 Material and equipment

- Human NF-κB Pathway Array Kit: Cat. ARY029, R & D Systems, Inc, Minneapolis, US.
- Human NF-κB Pathway Array: Cat. 894251, R & D Systems, Inc, Minneapolis, US.
- Array Buffer 1: Cat. 895477, R & D Systems, Inc, Minneapolis, US.
- Array Buffer 3: Cat. 895008, R & D Systems, Inc, Minneapolis, US.
- Array Buffer 6: Cat. 893573, R & D Systems, Inc, Minneapolis, US.
- Lysis Buffer 6: Cat. 895561, R & D Systems, Inc, Minneapolis, US.
- Wash Buffer Concentrate: Cat. 895003, R & D Systems, Inc, Minneapolis, US.
- Detection Antibody Cocktail, Human NF-κB Pathway Array: Cat. 894252, R & D Systems, Inc, Minneapolis, US.
- Streptavidin-HRP: Cat. 893019, R & D Systems, Inc, Minneapolis, US.
- Chemi Reagent 1: Cat. 894287, R & D Systems, Inc, Minneapolis, US.

- Chemi Reagent 2: Cat. 894288, R & D Systems, Inc, Minneapolis, US.
- 4-Well Multi-dish: Cat. 607544, R & D Systems, Inc, Minneapolis, US.
- Transparency Overlay Template: Cat. 607944, R & D Systems, Inc, Minneapolis, US.

2.5.2 NF-κB Protein Array

Cell Lysates

First wash the cells with PBS, of note, remove all residual PBS to ensure no residue remains before adding lysis solution. Keep the concentration of cells in the Lysis Buffer 6 to around 1 x 10^7 cells/ ml. Blast the cells with a pipette and allow them to shake evenly in the lysis buffer for 30 minutes at a controlled room temperature of 2-8°C. This will help the cells to lyse adequately. The lysed solution need to be centrifuged in a speed of 14,000 rpm for 5 minutes in a centrifuge at 4°C. After centrifugation, the supernatant need to be transferred into a new tube. A total protein assay is recommended to measure quantitation of sample protein concentrations. The maximum volume of lysate is 250 µl per array. The cell lysate should immediately be treated or dispensed and kept at low temperature normally ≤ -70° C

Reagent preparation

1. Human NF-κB Pathway Array - There are four nitrocellulose membranes in total, each membrane contains 45 different capture antibodies in the NF-κB pathway. The membranes need to be manipulated by forceps when in contact with them.

2. Detection Antibody Cocktail -One vial of lyophilized biotinylated antibody. Requires the addition of 100 µl of deionised or distilled water to reconstitute the Human NF-κB Pathway Detection Antibody Cocktail before use.

3. 1X Wash Buffer - First observe if there are crystals form in the concentrate reagent. If crystals are found to have formed in the concentrate, it should not be used directly

and the bottle needs to be heated to room temperature with mixing gently until the crystals are dissolved thoroughly before use. 1000 mL 1X Wash Buffer is prepared by adding 25X Wash Buffer concentrate40 mL to 960 mL of deionised or distilled water.

4. Chemi Reagent Mix - Chemical Reagent 1 and 2 must not be configured in advance. They should be configured within 15 minutes before use and need to be stored away from light. Each membrane requires 1 mL of the prepared mixture.

5. Array Buffer 3/6 – Mix same volumes of Array Buffer 3 and Array Buffer 6 to configure Array Buffer 3/6. When ready to use before preparation.

Assay procedure

1. Prepare the experimental sample, experimental tools and experimental reagents according to the steps that need to be prepared before the experiment.

2. Add 2.0 ml Array Buffer 3/6 per well to the 4-Well Multi-dish. The role of this procedure is to build up the block buffer in the experiment.

3. Gently remove the 4 membranes with the tweezers and place them in the wells of each of the 4-Well Multi-dish. Be gentle when handling with tweezers to prevent indentation. The number of each membrane should face upwards.

4. The 4-Well Multi-dish is covered and incubated on a shaker for one hour. During this process, ensure that the membrane is in solution and does not dry out.

5. The lysate was added to Array Buffer 1 to configure a 1.5 ml sample for the experiment.

6. Remove the 3/6 array buffer away from the 4-Well Multi-dish, then the prepared experimental samples from the previous step are added. Cover and place on a experimental shaker overnight for 4 °C.

7. Remove the 4 membranes with tweezers and rinse with 20 ml Wash Buffer carefully. Wash the 4-Well Multi-dish with distilled water and then dry them with a paper towel.

Each membrane should be immersed in the Wash Buffer and placed on the shaker for 10 minutes. This step needs to be repeated 2 times for a total of 3 rinses.

8. Add 1.5 ml of the prepared Detection Antibody Cocktail per well of the 4-Well Multi-dish.

9. Erase the 4 membranes with tweezers and place into the4-Well Multi-dish prepared with the Detection Antibody Cocktail. After covering, incubate on a shaker for 1 hour at room temperature.

10. Repeat step 7 to wash the membranes and culture 4-Well Multi-dish.

11.Add 2.0 ml of prepared diluted Streptavidin-HRP per well of each 4-Well Multi-dish. Place 4 membranes into the 4-Well Multi-dish using tweezers, cover and incubate on a shaker for half an hour at room temperature.

12. Repeat step 7 to wash the membranes and culture 4-Well Multi-dish.

13. Add 1 mL of the ready Chemi Reagent Mix equally onto each membrane with pipette. Make sure that the reagents are evenly distributed and that no air bubbles appear. If air bubbles are found, remove them with tweezers. Stand for 1 minute at room temperature.

14. Drain the excess Chemi Reagent Mix. Place a paper towel on the top side of the transparent plastic sheet protector holding the membrane and squeeze the surplus Chemi Reagent Mixout gentally. This process should remove as much of the Chemi Reagent Mix as possible so as not to interfere with the next step and the results of the experiment.

15. Remove the 4 membranes and gently blot up any residual Chemi Reagent Mix with a paper towel.

16. Leave the 4 membranes on the bottom side of transparent plastic sheet protector and cover with cling film, observe if any air bubbles are produced and remove them if they are. This process should be fully covered with cling film.

17.The four membranes are exposed to X-rays in sequence for a 10 minute exposure time. The exposure time is determined by the exposure results.

18.Acquisition of dot blot images can be obtained with using the Chemidoc MP Imaging system (Bio Rad, Hercules, CA). Observe whether the control group appears, if it does not appear, the experiment is invalid. The acquired dot blot images were analyzed using ImageJ software for optical density and the results of the experiment were obtained.

2.6 Statistics

The analysis of the data obtained from this experiment was carried out using the statistical software SPSS 21.0 (IBM Corporation, NY, USA). To test for normality, Shapiro-Wik's test was used. As the data is on normal distribution, differences between the experimental and control groups were compared using a two-tailed unpaired Student's t-test. Statistical significance was considered to be positive, when the p-value was <0.05.

3 Results

3.1IL-1β expression in lung cancer cells and macrophages mono-cultured supernatants

Interleukin-1β expression was detected in H838, H1339, H1650, H1975 and macrophage supernatants; IL-1β was not detected in monoculture lung cancer cell supernatants. However, it was detected in macrophage supernatants at a concentration of 0.33 pg/ml. (figure 3)

Figure 3

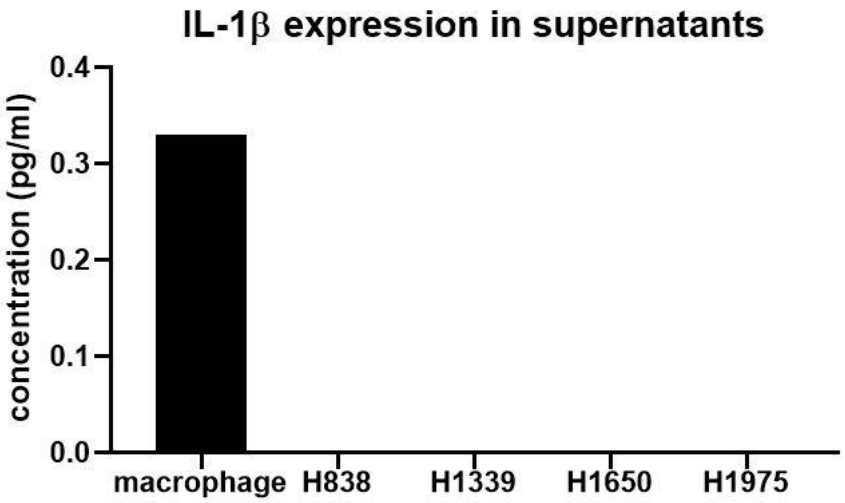

Figure 3: The concentration of IL-1β in supernatants of lung cancer cell lines and macrophages In the macrophage supernatant, the concentration was 0.33 pg/ml; however, in supernatants of H838, H1339, H1650 and H1975 cells, IL-1β was not detected.

3.2 IL-1β expression in a supernatant of co-cultured lung cancer cells and macrophages

Lung cancer cells and macrophages were placed in a 5:1 ratio in the temperature is 37 and CO_2 concentration is 5% incubator for 7 days. The supernatant of the cell culture was collected once a day at a fixed time point during this consecutive 7-day period.

3.2.1 IL-1β expression in co-culture of H838 cells and macrophages

A co-culture of H838 and macrophages was observed with a microscope every 24 hours. We found that H838 was predominant. After seven days, all the macrophages had died, and H838 had grown and covered the bottom of the flask. The expression of IL-1β started on day 0, reaching a concentration of 0.3 pg/ml, close to that of the macrophage monoculture (0.33 pg/ml). With the interaction of H838 and macrophages, IL-1β expression rapidly increased, reaching its highest concentration of 0.764 pg/ml on the first day. After apoptosis of the macrophages began, the number of macrophages decreased, as did the concentration of secreted IL-1β. When all the macrophages had died, the IL-1β concentration dropped to a minimum of 0.03 pg/ml on the seventh day. (figure 4)

Figure 4

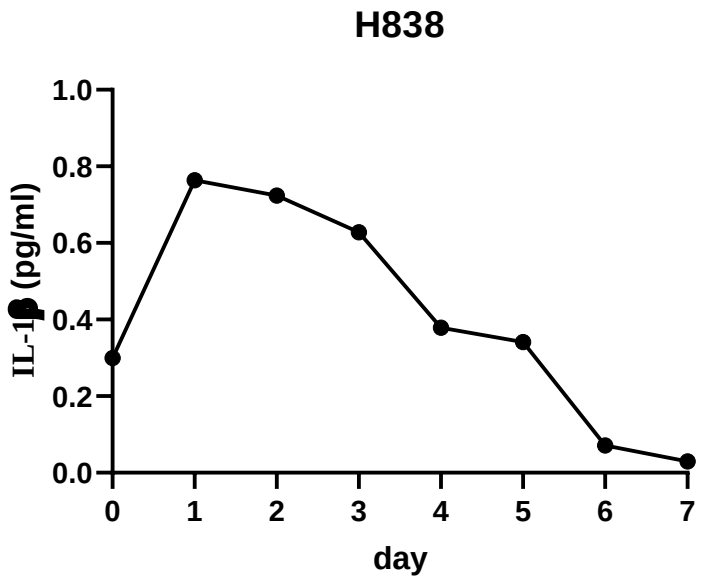

Figure 4: The concentration of IL-1β in a supernatant of co-cultured H838 and macrophages over seven consecutive days The expression of IL-1β started on day 0, reaching a concentration of 0.3 pg/ml, and rapidly increased to reach its highest concentration of 0.764 pg/ml on the first day. The concentration then gradually declined, dropping to 0.03 pg/ml on the seventh day.

3.3.2IL-1β expression in co-culture of H1339 cells and macrophages

A co-culture of H1339 and macrophages was observed with a microscope every 24 hours. We found that H1339 was predominant. After seven days, all the macrophages had died, and H1339 had grown and covered the bottom of the flask. The expression of IL-1β started on day 0, reaching a concentration of 0.287pg/ml, close to that of the macrophage monoculture (0.33 pg/ml). With the interaction of H1339 and macrophages, IL-1β expression rapidly increased, reaching its highest concentration of 1.479pg/ml on the second day. After apoptosis of the macrophages began, the number of macrophages decreased, as did the concentration of secreted IL-1β. When all the macrophages had died, the IL-1β concentration dropped to a minimum of 0.02pg/ml on the seventh day. (figure 5)

Figure 5

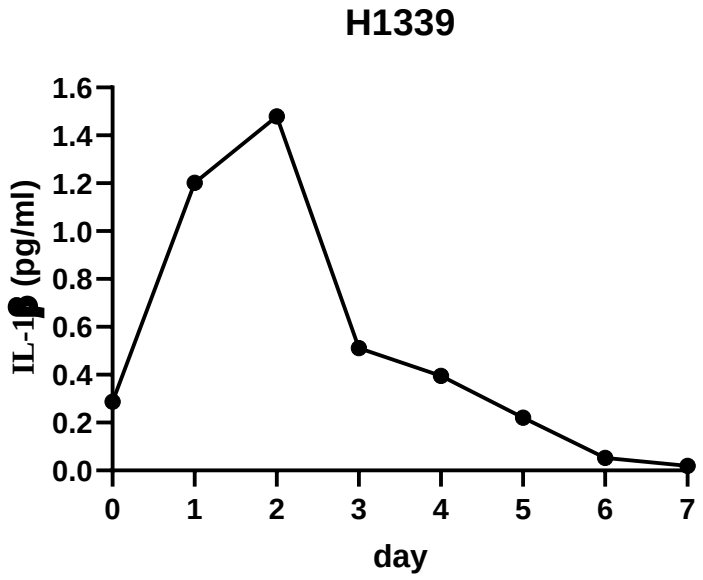

Figure 5: The concentration of IL-1β in a supernatant of co-cultured H1339 and macrophages over seven consecutive days The expression of IL-1β started on day 0, reaching a concentration of 0.287pg/ml, and rapidly increased to reach its highest concentration of 1.479pg/ml on the second day. The concentration then gradually declined, dropping to 0.02pg/ml on the seventh day.

3.2.3IL-1β expression in co-culture of H1650 cells and macrophages

A co-culture of H1650 and macrophages was observed with a microscope every 24 hours. We found that H1650 was predominant. After seven days, all the macrophages had died, and H1650 had grown and covered the bottom of the flask. The expression of IL-1β started on day 0, reaching a concentration of 0.321pg/ml, close to that of the macrophage monoculture (0.33 pg/ml). With the interaction of H1650 and macrophages, IL-1β expression rapidly increased, reaching its highest concentration of 1.18pg/ml on the first day. After apoptosis of the macrophages began, the number of macrophages decreased, as did the concentration of secreted IL-1β. When all the macrophages had died, the IL-1β concentration dropped to a minimum of 0.084pg/ml on the seventh day. (figure 6)

Figure 6

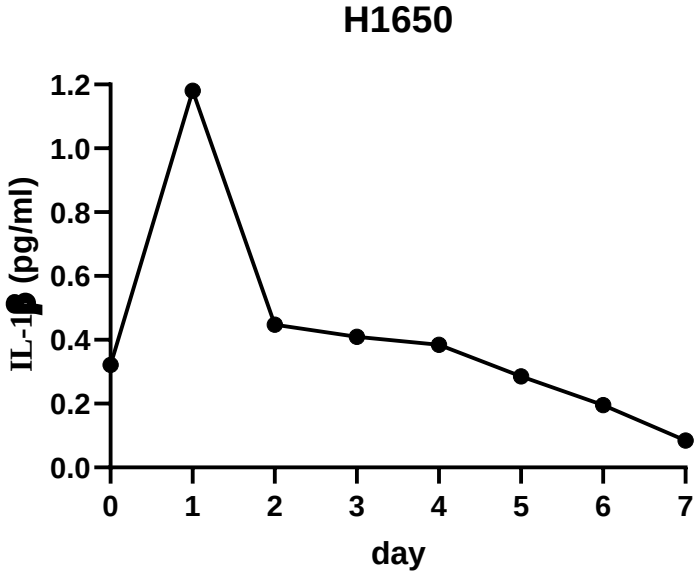

Figure 6: The concentration of IL-1β in a supernatant of co-cultured H1650 and macrophages over seven consecutive days The expression of IL-1β started on day 0, reaching a concentration of 0.321pg/ml, and rapidly increased to reach its highest concentration of 1.18pg/ml on the first day. The concentration then gradually declined, dropping to 0.084pg/ml on the seventh day.

3.2.4IL-1β expression in co-culture of H1975 cells and macrophages

A co-culture of H1975 and macrophages was observed with a microscope every 24 hours. We found that H1975 was predominant. After seven days, all the macrophages had died, and H1975 had grown and covered the bottom of the flask. The expression of IL-1β started on day 0, reaching a concentration of 0.342pg/ml, close to that of the macrophage monoculture (0.33 pg/ml). With the interaction of H1975 and macrophages, IL-1β expression rapidly increased, reaching its highest concentration of 0.678pg/ml on the first day. After apoptosis of the macrophages began, the number of macrophages decreased, as did the concentration of secreted IL-1β. When all the macrophages had died, the IL-1β concentration dropped to a minimum of 0.102 pg/ml on the seventh day. (figure 7)

Figure 7

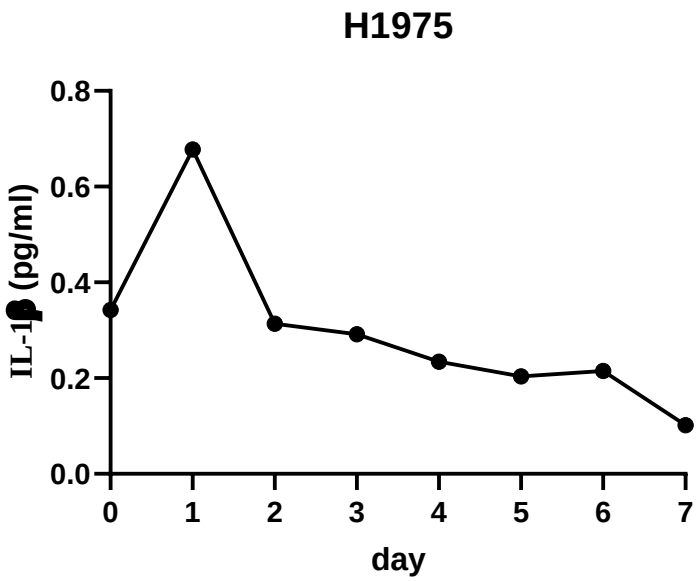

Figure 7: The concentration of IL-1β in a supernatant of co-cultured H1975 and macrophages over seven consecutive days The expression of IL-1β started on day 0, reaching a concentration of 0.342pg/ml, and rapidly increased to reach its highest concentration of 0.678pg/ml on the first day. The concentration then gradually declined, dropping to 0.102pg/ml on the seventh day.

3.3 IL-1R1expressionin lung cancer cell lines and macrophages lysates

Interleukin-1β receptor 1 expression was detected in H838, H1339, H1650, H1975 and macrophage lysates. The receptor on macrophages is higher expressed than on lung cancer cell lines. It was detected in macrophage lysates at a concentration of 857.56pg/ml. And it was detected in H838, H1339, H1650 and H1975cellslysates at a concentration of224.46pg/ml,310.18pg/ml,269.53pg/ml and 328.91pg/ml respectively. (figure 8)

Figure 8

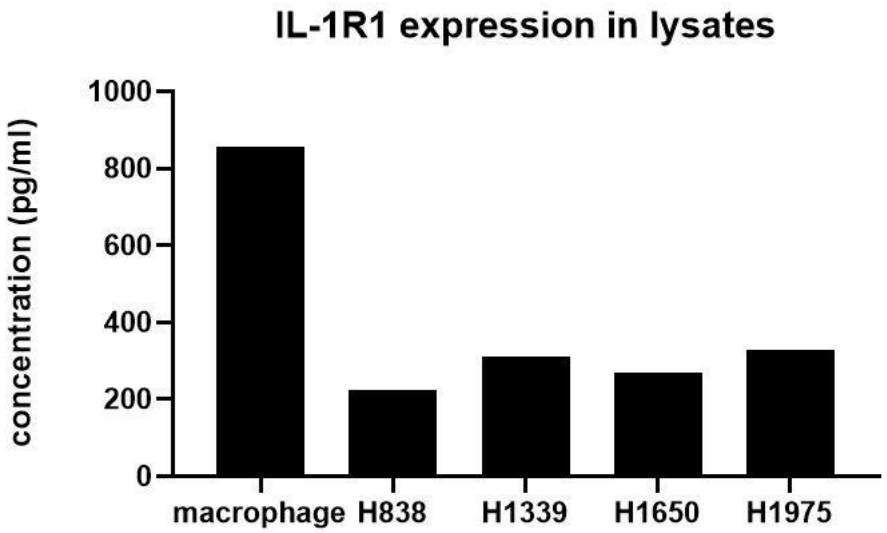

Figure 8: The concentration of IL-1R1 in the lysates of lung cancer cells and macrophages In the macrophage lysate, the concentration was 857.56pg/ml. In the H838, H1339, H1650 and H1975 cells, the concentration was 157.26pg/ml,224.46pg/ml,310.18pg/ml ,269.53pg/ml and 328.91 pg/ml respectively.

3.4 NF-κB signalling pathway-related protein expression with treated and untreated IL-1β

3.4.1NF-κB signalling pathway-related protein expression in H838 cells

To detect the alteration of IL-1β in the NF-κB pathway proteins in H838cells, H838 cells were treated with IL-1β in the concentration of 2 pg/ml for 24 hours; untreated H838 cells were the control group. In the IL-1β activation pathway, we selected 10 major proteins (IκBα, IκBε, IKKα, IKKβ, IKKγ, NFκB1, NFκB2, RelA, RelA (pS529) and c-Rel) (figure 9). With the H838 cells, we used statistical analysis to compare two sets of data and found that the expressions of IκBα, NFκB2 and RelA had statistical significance ($p < 0.05$). (fable 1)

Figure 9A

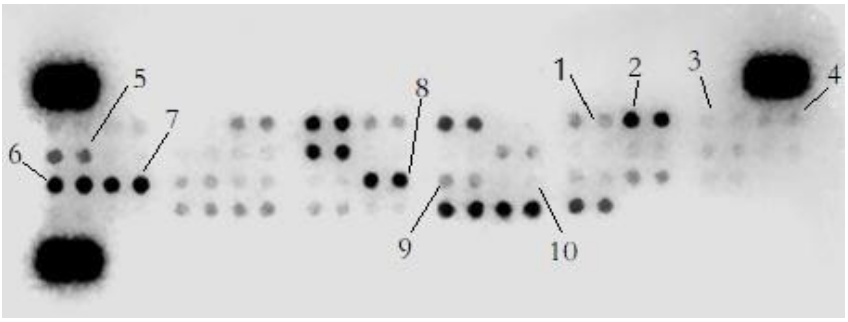

Figure 9B

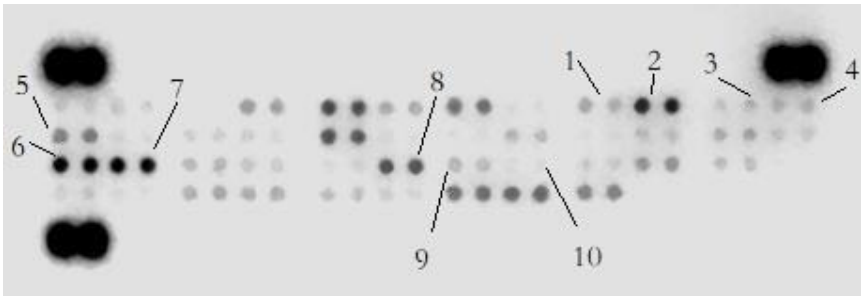

Figure 9C

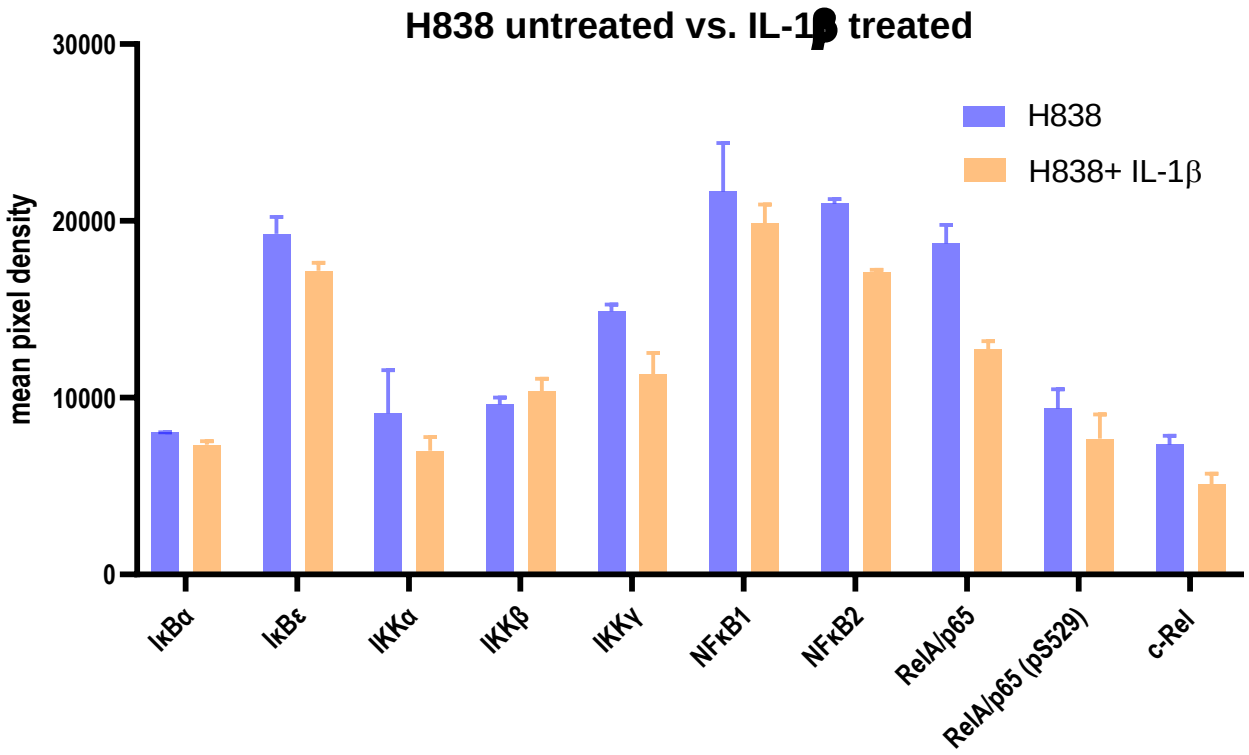

Figure 9: Protein expression in H838 cells with untreated and treated IL-1βH838 cells were untreated (Figure 9A) and treated (Figure 9B) with 2 pg/ml IL-1β for 24 hours (250 µg lysate, 10 minutes exposure). In the exposed photos, the numbers 1 to 10 represent IκBα, IκBε, IKKα, IKKβ, IKKγ, NFκB1, NFκB2, RelA, RelA (pS529) and c-Rel, respectively. Protein array analysis showed that in the H838 cells, with IL-1β treated, the expression of IκBα, IκBε, IKKα, IKKγ, NFκB1, NFκB2, RelA, RelA (pS529) and c-Rel decreased and expression of IKKβ increased compared with the untreated sample (n = 2).

Table 1

proteins	**untreated**	**treated**	***p* value**
IκBα	8021.5	7346	0.03701
IκBε	19262	17179	0.110858
IKKα	9128.5	6995	0.358
IKKβ	9636.5	10357.5	0.3363
IKKγ	14886	11332	0.056879
NFκB1	21661.5	19854.5	0.4767
NFκB2	20975	17118	0.002509
RelA/p65	18733	12728	0.01756
RelA/p65 (pS529)	9398.5	7689	0.297
c-Rel	7360	5107	0.053914

Table 1: Protein pixel density and *p* value for H838 cells with untreated and treated IL-1β.

3.4.2NF-κBsignalling pathway-related protein expression in H1975 cells

To detect the alteration of IL-1β in the NF-κB pathway proteins in H1975cells, H1975 cells were treated withIL-1β in the concentration of 2 pg/ml for 24 hours; untreated H1975 cells were the control group. In the IL-1β activation pathway, we selected 10 major proteins (IκBα, IκBε, IKK α , IKKβ, IKKγ, NFκB1, NFκB2, RelA, RelA (pS529) and c-Rel) (figure 10). With the H1975 cells, we used statistical analysis to compare two sets of data and found that the expressions of IκBα, IκBε, IKKβ, NFκB1, NFκB2 and RelA(pS529) had positive significance in statistics ($p < 0.05$). (table 2)

Figure 10A

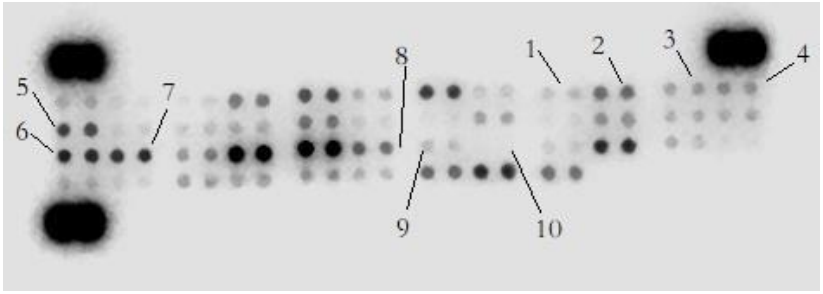

Figure 10B

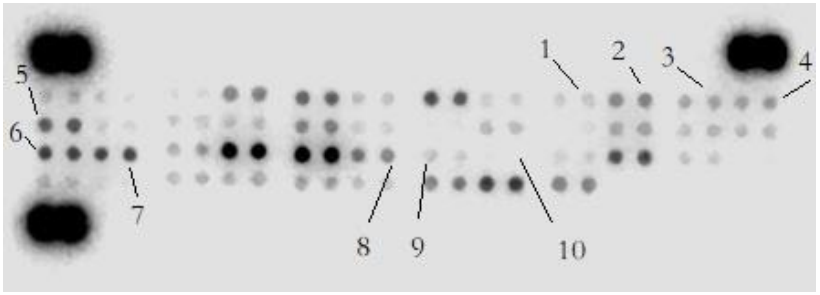

Figure 10C

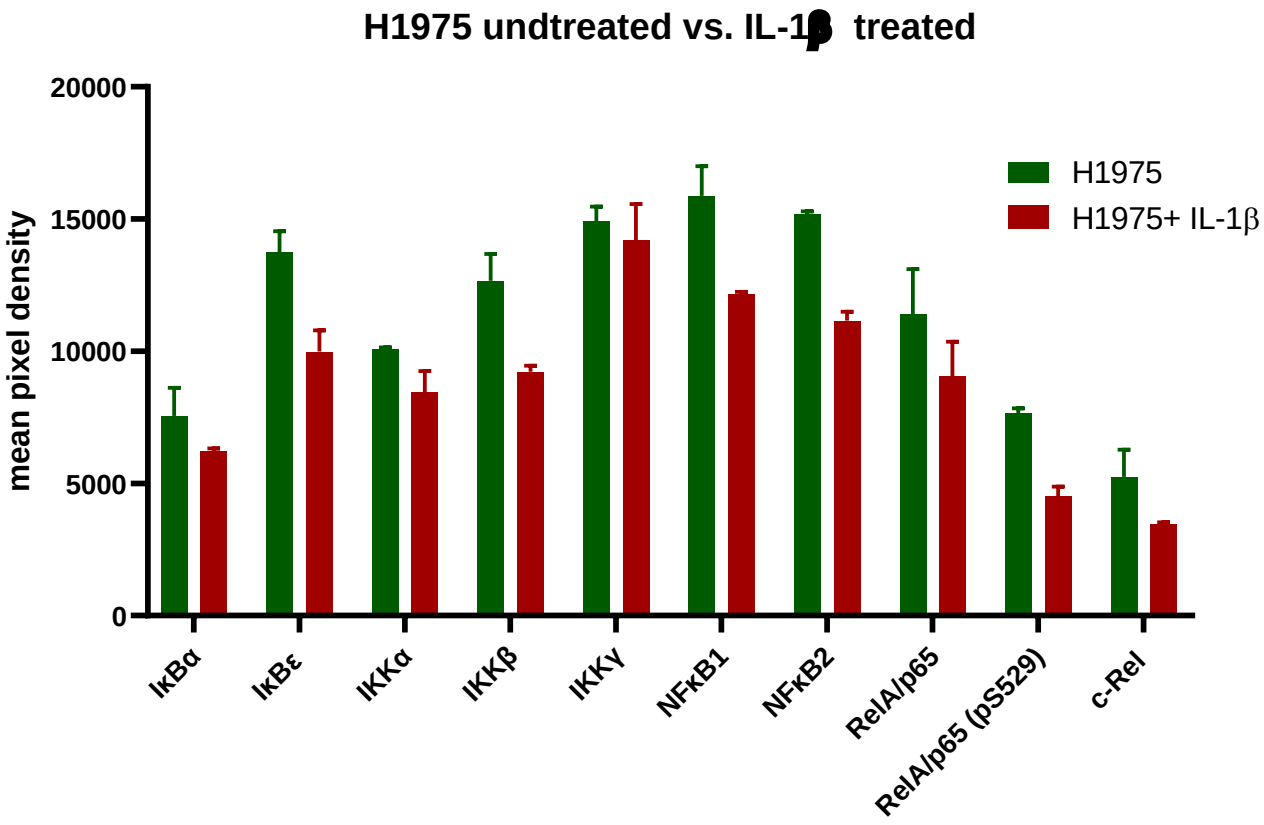

Figure 10: Protein expression in H1975 cells with untreated and treated IL-1β

H1975 cells were untreated (Figure 10A) and treated (Figure 10B) with 2 pg/ml IL-1β for 24 hours (250 µg lysate, 10 minutes exposure). In the exposed photos, the numbers 1 to 10 represent IκBα, IκBε, IKKα, IKKβ, IKKγ, NFκB1, NFκB2, RelA, RelA (pS529) and c-Rel, respectively. Protein array analysis showed that in the H1975 cells, with IL-1β treated, the expression of IκBα, IκBε, IKKα, IKKβ, IKKγ, NFκB1, NFκB2, RelA, RelA(pS529) and c-Rel decreased compared with the untreated sample (n = 2).

Table 2

proteins	**untreated**	**treated**	***p* value**
IκBα	7553	6235	0.02236
IκBε	13751	9987	0.041751
IKKα	10098	8452	0.09887
IKKβ	12662	9221	0.04524
IKKγ	14906	14206	0.569588
NFκB1	15873.5	12154.5	0.04317
NFκB2	15179.5	11157	0.00384
RelA/p65	11412	9052.5	0.2588
RelA/p65 (pS529)	7649	4525	0.008103
c-Rel	5247	3485	0.135568

Table 2: Protein pixel density and *p* value for H1975 cells with untreated and treated IL-1β.

4 Discussion

4.1 Effect of IL-1β on lung cancer cell lines

Ridker et al. report found that IL-1β blocking antibody canakinumab reduce the incidence and mortality of lung cancer [77]. To address the underlying mechanism, we established lung cancer co-cultures to investigate the interactions between lung cancer cells and immunocyte macrophages related to IL-1β expression.

Interleukin-1β can be regulated and expressed through a variety of inflammatory mediators and is the main inflammatory cytokine secreted by macrophages, which play a very important role in the regulation of inflammatory mediators [52, 53]. Voronov E et al. demonstrated that IL-1β is the pro-inflammatory cytokine released by the tumour microenvironment and involved in the process of tumour formation, and acts a critical position in the regulation of tumours in several ways[78-83]. The aberrant expression of IL-1β is thought to promote tumour growth and metastasis. Wang L et al. found that NSCLC patients had significantly higher expression of IL-1β in their peripheral blood serum compared to healthy individuals. Moreover, IL-1β secreted by the alveolar macrophages of patients with lung cancer was much higher than that secreted by peripheral blood mononuclear cells of the same patients, showing its role in tumour development[54].

In this study, we found that when macrophages were co-cultured with lung cancer cells, the lung cancer cells enhanced IL-1β expression in macrophages.

The process of tumour development often involves infiltration of inflammatory cells such as macrophages, which are also an important feature of tumours. Tumor-associated macrophages are macrophages that have infiltrated the tumour tissue, and their number is closely related to the poor prognosis of the tumour [50]. Activated TAMs infiltrate the tumour microenvironment and produce cytokines and chemokines through autocrine and paracrine methods to regulate tumour growth [51]. In mice deficient in macrophages, tumour growth and metastasis are almost completely inhibited [84].

Many macrophages infiltrate all subtypes of lung cancer, while normal lung tissue contains only a few macrophages [85]. The results of this study show that macrophages can significantly promote the proliferation of H838, H1339, H1650 and H1975 cells.

Macrophages can produce assorted cytokines that play a critical function in tumour proliferation and development, such as interleukin and tumour necrosis factor; IL-1β is an important pro-inflammatory factor expressed by macrophages. Several studies have found that IL-1β can promote the development of lung cancer, colorectal cancer and other tumours [52, 86].Its mechanism includes promotion of tumour cell growth by activating the Wnt signalling pathway of colorectal cancer; IL-1β has an angiogenic function and can increase the formation of blood vessels around lung tumours [55].It promotes the development of lung cancer by inhibiting the microRNA-101/Lin28B pathway of lung cancer cells [86]. In this study, lung cancer cells and macrophages were directly co-cultured to detect IL-1β expression in macrophages and tumour cells. The results indicated that when lung cancer cells were co-cultured with macrophages, IL-1β expression in macrophages was meaningfully enhanced and higher than that in a lung cancer cell monoculture. This suggests that the main source of IL-1β expression and secretion during co-culture was macrophages. Yurong et al. used IL-1β neutralising antibody to inhibit IL-1β. The results showed that the ability of macrophages to boost the proliferation of lung cancer cells was significantly reduced, confirming that IL-1β released by macrophages is a key factor in promoting the proliferation of tumour cells [87].

In summary, the results of this study showed that in co-cultures model, lung cancer cells can promote the secretion of IL-1β by macrophages, resulting in elevated expression. And the secreted IL-1β could also raise the proliferation of lung cancer cells. In future research, an accidental discovery related to lung cancer in the CANTOS trial may lead to a treatment strategy or even a prevention plan for high-risk groups.

4.2 Effect of NF-κB Pathway agonist on Lung Cancer Cell lines

The NF-κB pathway acts a critical function in tumour development and has been extensively studied, particularly in the regulation of immune responses and inflammation in tumour tissue. the NF-κB pathway is involved in numerous regulatory processes that involve the expression of many gene products that play acritical role in many key aspects of tumours, such as tumour proliferation, tumour migration and tumour apoptosis. As more and more studies on malignant tumours are conducted, more and more articles suggest that the NF-κB pathway is activated in malignant tumours [88].

To explore the role of IL-1β in the NF-κB pathway in lung cancer cells, we performed an NF-κB protein array. The protein expression of NF-κB pathway components IκBα, IκBε, IKKα, IKKβ, IKKγ, NFκB1, NFκB2, p65, p65 (pS529) and c-Rel were investigated in the study. In H838 and H1975 cells, all the proteins were expressed substantially. Protein array analysis showed decreased expression of the IKK family (IKKα, IKKβ and IKKγ), IκB family (IκBα, IκBε) and NF-κB family members (NFκB1, NFκB2, p65, p65 (pS529) and c-Rel) in H838 and H1975 cells.

The NF-κB protein is usually in a homo/heterodimer form; it is inactive in the cytoplasm due to the formation of a trimer complex with the inhibitory protein IκB. When the upstream signalling factor IL-1β binds to the receptor on the cell membrane surface, the receptor conformation changes and transmits the signal to the IKK kinase, which in turn phosphorylates the IκB protein and dissociates it from the trimer. This dissociation is due to the phosphorylation of IKK at serine 32 and serine 36, which results in the degradation of the protein. IKK is a complex containing IKKα, IKKβ and IKKγ subunits [89]. Inactivation or degradation of the IκBα protein develop to an alteration in the conformation of the protein and, as a result of this change in conformation, NF-κB also changes from its former inactive bound state to a free activated formation and exists. When NF-κB gains activity, it can easily cross the nuclear membrane into the nucleus, then bind to particular DNA sequences in the

promoter region of target genes and activate transcription [90, 91]. The IκB and IKK play a critical role in the pathway activation. Due to their phosphorylation, their expression levels are reduced [92-94].

Our study found that activation of the NF-κB pathway on lung cancer cells with IL-1β resulted in a decline in the concentration of many proteins on the NF-κB pathway. The effect of IL-1β on NF-κB pathway activity is mediated by activation of the downward transmission of the pathway caused by the NF-κB pathway, rather than by altering the concentration of downstream proteins. After phosphorylation of the activated NF-κB dimer, NF-κB passes through the nuclear membrane into the nucleus and binds to specific genes to function. In a study by Jing et al. NF-κB activation by IL-1β was confirmed by electrophoretic mobility shift assay (EMSA) [95]. Ling et al. confirmed NF-κB activation based on an increase in nuclear NF-κB and a decrease in cytosolic NF-κB [96].

NF-κB not only participates in cell cycle regulation, autophagy, aging, apoptosis, inflammatory and immune responses and other pathophysiological processes but also acts a critical position in the occurrence, development, infiltration, metastasis and treatment of tumours.

In H838 and H1975 cells, activated NF-κB was mainly responsible for promoting the value-added process in lung cancer cells by governing the expression of proliferation-associated proteins. Activated NF-κB can alter the accelerated lung cancer cell cycle transition or shorten the cell cycle, especially from G1 to S phase and G2 to M phase, shortening the original cell cycle and thus accelerating tumour growth[73, 97].In H838 cells, NF-κB inhibitors reduce cells in G0 and G1 phases and escalate cells in S phase[97].In H1975 cells, blocking the NF-κB pathway can protect H1975 cells from lipopolysaccharide-induced apoptosis and inflammatory damage [98].In H1975 cells, eugenol inhibits NSCLC by repressing expression of NF-κB-regulated TRIM59[99].In lung cancer A549 cells, activated NF-κB downregulated the expression of Bax, APAF1, Fas, Bak and other pro-apoptotic proteins and upregulated the expression of Bcl-2, XIAP, BCL-XL and other anti-apoptotic proteins by inhibiting the activity of caspase-

9/caspase-3 to weaken the apoptosis of lung cancer cells [100]. Activating NF-κB can also reduce the apoptosis of lung cancer cells by inhibiting the Fas-mediated extracellular apoptosis pathway [73]. In A549 cells, the expression level of NF-κB meaningfully correlated with the expression levels of VEGF, COX-2 and IL-8. Highly expressed NF-κB promoted neovascularization in lung cancer tissue by upregulating the expression of its downstream genes (VEGF, COX-2 and IL-8)[101].In the Lewis lung cancer mouse model, NF-κB promotes neovascularization of lung cancer by upregulating the expression of angiogenesis-related factors such as bFGF, VEGF and PD-ECGF[102].In A549 cells, NF-κB promotes the epithelial-mesenchymal transformation of NSCLC cells and the formation of tumour stem cells by upregulating the expression of the epithelial-to-mesenchymal transition (EMT) master switch transcription factors TWIST1, ZEB2 and SNAIL2, thereby promoting the invasion and metastasis of NSCLC[74].

Nuclear factor κB plays a critical role in lung cancer, suggesting that the NF-κB pathway can be used as a new direction for treating lung cancer. Currently, most studies focus on inhibiting the activation of IKK (IKK2 inhibitor) and blocking the degradation of IκBα (proteasome inhibitor). The proteasome inhibitor bortezomib apply an anti-tumour effect by blocking the degradation of IκBα and inhibiting the activity of NF-κB. After a phase II clinical study, the efficacy of bortezomib in the treatment of NSCLC patients remained uncertain, and large-scale studies are needed to confirm its efficacy [103, 104].Allyson et al. showed that IL-1β is a targetable, pro-tumorigenic mediator that contributes to lung tumour resistance to bortezomib[105]; IL-1β inhibitors reduce the resistance of lung tumours to NF-κB inhibitors and provide new ideas for treating lung cancer.

Application of NF-κB inhibitors against tumours is a promising adjuvant therapy. The disadvantage is that the current NF-κB inhibitors are not tissue-specific, and clinical application may cause damage to tissues and organs and increase the probability of opportunistic infection. With the development of gene therapy technology, accurate regulation of NF-κB activity could potentially become a new strategy for clinical

treatment of inflammation-related diseases and tumours.

4.3 Outlook

Lung cancer is the primary common cancer in the world and one of the most deadly malignancies caused by tumours. Recently a large trial of canakinumab in cardiac patients described lower lung cancer incidence in patients treated with canakinumab compared to controls. This finding is the basis for ongoing clinical trials of canakinumab in lung cancer. Canakinumab may have a prophylactic effect as one of the treatments for lung cancer, as well as potentially targeting people with a high incidence of lung cancer for prevention. It may be a fresh option for the treatment of lung cancer in the future.

Inflammatory cytokine IL-1β is the main driving factor of the tumour inflammatory response and dominates the tumour microenvironment. It can strongly induce the activity of myeloid-derived inhibitory cells and increase the secretion of tumour-promoting cytokines, thereby promoting tumour formation and invasion of the tumour microenvironment. Therefore, IL-1β inhibitors may target tumour-related inflammatory responses, reduce immunosuppression and promote anti-tumour activity in lung cancer cells. There is a need for a better understanding of IL-1β in lung cancer cellular mechanisms, including functional evidence for the mechanism by which IL-1β promotes cancer cell proliferation cancer cells and escapes from conventional cancer therapy. Cancer-specific markers of IL-1β must be found and methods for in vivo detection of IL-1β must be established to use the status of IL-1β in cancer as an indicator of the likelihood of recurrence and as an indicator of treatment efficacy. Thus, the incorporation of anti-IL-1β components into therapeutic regimens could expand the field of cancer treatment. A new therapeutic strategy may be to combine conventional drugs that target tumours with anti-IL-1β components (canakinumab), thereby improving treatment effect. As the IL-1β inhibitor canakinumab has entered clinical trials in lung cancer, our findings support the value of IL-1β inhibitors in lung cancer

research, particularly in the prevention and conservative treatment of high-risk groups. IL-1β inhibitors need to be studied in more depth, particularly in patients with lung cancer, and will have some clinical value, perhaps as a powerful treatment for lung cancer in the future.

Additionally, further research into the NF-κB pathway activation mechanism in lung cancer is required, for instance, the interaction between proteins in the NF-κB pathway and those in other pathways, since our work showed that NF-κB pathway agonist IL-1β shows a pro-tumour effect in lung cancer. It is necessary to construct a network to increase understanding of the IL-1β and NF-κB pathways in relation to tumorigenesis and furthermore to establish a new cancer therapy. While NF-κB pathway inhibitors have been used in clinical research, they can also affect the function of normal cells. Future research should investigate how to use the inhibitors to accurately target the NF-κB pathway in tumour tissues or tumour cells. This will provide new programs for the adjuvant treatment of lung cancer.

5 Conclusion

In this study, lung cancer cells H838, H1339, H1650 and H1975 promoted IL-1β secretion by macrophages. In a lung cancer and macrophages co-culture leads to higher expression of IL-1β than lung cancer cell monoculture. We found no IL-1β expression in the supernatant of cultured lung cancer cells. However, we found that IL-1β expression occurred in a macrophage supernatant. This confirms that the IL-1β we detected is only secreted by macrophages.

Lung cancer cells promote IL-1β secretion by macrophages and are related to the number of macrophages. In a co-culture, the amount of secreted IL-1β is related to the number of macrophages. In the co-culture, daily observation showed that the number of macrophages gradually declined, and IL-1β secretion decreased accordingly.

IL-1β activates the NF-κB pathway and affects the expression of proteins on the pathway in H838 and H1975 cells.In H838 cells, IκBα, NFκB2 and RelAexpression was found to be down-regulated. In H1975 cells, IκBα, IκBε, IKKβ, NFκB1, NFκB2 and RelA (pS529) expression was found to be down-regulated.

Lung cancer cells can stimulate macrophages to increase IL-1β secretion, which can activate the NF-κB pathway in these cells, producing a more favourable growth environment. These interactions should be considered when studying and modeling the expression of IL-1β as a potential therapeutic target in lung cancer.

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