The role of TRPA1 in pulmonary pathology

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SVEUČILIŠTE U RIJECI
ODJEL ZA BIOTEHNOLOGIJU
Preddiplomski sveučilišni studij
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Andrej Kuprešak

Uloga TRPA1 kanala u patologiji pluća

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Mentor: doc.dr.sc. Christian Reynolds

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Summary

The goal of this work is to better understand how lung disorders including asthma and chronic obstructive pulmonary disease (COPD) are affected by the TRPA1 channel, a crucial molecular sensor. The TRPA1 channel is involved in controlling inflammation, pain perception, and the respiratory system's reaction to damaging stimuli. It is activated by a variety of chemical irritants. The desire to find novel treatment targets and get a deeper understanding of the molecular mechanisms behind the course of lung illness is what motivates this research's scientific setting.

The study entails a careful review of the body of literature already in existence, with an emphasis on the findings of knockout (KO) studies, which employed mice deficient in the TRPA1 gene to investigate its function. The precise functions of the TRPA1 channel in the pathophysiology of lung diseases have been made clearer by these models. Furthermore, the possibility of pharmacologically blocking the TRPA1 channel is taken into consideration as a potential treatment approach.

According to the research, the TRPA1 channel plays a significant role in several processes that lead to inflammation and lung tissue destruction. As a result, blocking this channel may lessen symptoms and delay the course of conditions like asthma and COPD. The study's conclusion highlights the need for ongoing research to maximize therapeutic agent selectivity and gain a deeper understanding of the roles played by the TRPA1 channel in various tissues, as well as the potential for new avenues in the development of targeted therapies for lung diseases
Key Words: TRPA1 Channel; Lung Diseases; Lung Cells; Knockout Experiments; Pharmacological Inhibition

Sažetak

Ovo istraživanje usmjereno je na razumijevanje uloge TRPA1 kanala, ključnog molekularnog senzora, u plućnim bolestima poput astme i kronične opstruktivne plućne bolesti (KOPB). TRPA1 kanal, poznat po svojoj aktivaciji različitim kemijskim iritantima, sudjeluje u regulaciji upale, osjetu boli i reakcijama na štetne podražaje u dišnom sustavu. Znanstveni kontekst ovog rada temelji se na potrebi za dubljim razumijevanjem molekularnih mehanizama koji stoje iza progresije plućnih bolesti i na identificiranju novih terapijskih ciljeva.

Istraživanje koristi pristup koji uključuje kritičku analizu postojeće literature, s posebnim naglaskom na rezultate knockout pokusa u kojima su miševi bez TRPA1 gena korišteni za proučavanje njegove funkcije. Ovi modeli omogućili su otkrivanje specifičnih uloga TRPA1 kanala u patofiziologiji plućnih bolesti. Uz to, razmatra se farmakološka inhibicija TRPA1 kanala kao potencijalna terapijska strategija.

Rezultati pokazuju da je TRPA1 kanal uključen u brojne ključne procese koji pridonose upali i oštećenju plućnog tkiva, te da inhibicija ovog kanala može smanjiti simptome i progresiju bolesti kao što su astma i KOPB. Zaključci rada sugeriraju da daljnje istraživanje TRPA1 kanala može otvoriti nove puteve u razvoju ciljanih terapija za plućne bolesti, te ističu potrebu za daljnjim istraživanjima kako bi se optimizirala selektivnost terapijskih sredstava i bolje razumjele funkcije ovog kanala u različitim tkivima.

Ključne riječi: TRPA1 kanal; Plućne bolesti; Plućne stanice; knockout pokusi; Farmakološka inhibicija

List of abbreviations

TRPA1 -Transient receptor potential ankyrin 1 channel

CO_2 -Carbon dioxide

COPD - Chronic obstructive pulmonary disease

PPAR - Peroxisome proliferator-activated receptor

PGE2 - Prostaglandin E2

CYP450 - Cytochrome P450

NO- Nitric oxide

NF-kB - Nuclear factor kappa-light-chain-enhancer of activated B cells

MAPK - Mitogen-activated protein kinase

IKK - IκB kinase

IL - Interleukine

TNF - Tumor necrosis factor

TGF-β - Transforming growth factor beta

ERK - Extracellular signal-regulated kinase

JNK - c-Jun N-terminal kinase

STAT - Signal transducer and activator of transcription

cAMP - Cyclic adenosine monophosphate

cGMP - Cyclic guanosine monophosphate

eNOS - Endothelial nitric oxide synthase

sGC - Soluble guanylate cyclase

RTP - Receptor-transducer protein

MMP - Matrix metalloproteinase

CGRP - Calcitonin gene-related peptidei

SP - Substance P

BRS-3 - Bombesin receptor subtype 3

CB1 - Cannabinoid receptor type 1

CB2 - Cannabinoid receptor type 2

TRPV1 - Transient receptor potential vanilloid 1

TRPM8 - Transient receptor potential melastatin 8

DRG - Dorsal root ganglion

TDM - Tissue distribution model

PCR - Polymerase chain reactio

Table of Contents

1. ABOUT LUNGS AND TRPA CHANNELS

A sizable family of ion channels known as transient receptor potential (TRP) channels is essential to the body's capacity to detect and react to a wide range of internal and external stimuli. As molecular sensors, these channels control a wide range of physiological functions, including as osmoregulation, pain perception, temperature feeling, mechanical force sensing, and reactions to various chemical stimuli. The TRPA (Transient Receptor Potential Ankyrin) subfamily of TRP channels is distinguished from the other subfamilies by virtue of its special characteristics. As a chemosensor that reacts to a wide range of chemical irritants and physiological changes, the TRPA1 channel stands out within this group and is important for several pathological events[1].

1.1. TRPA1 channel: overview and operation

Because it plays a role in pain perception and chemical detection, as well as the body's reaction to damaging stimuli including irritants and inflammatory mediators, the TRPA1 channel is particularly fascinating. Ankyrin repeats, which are essential for mechanical signaling and protein-protein interactions, are abundant in the structural makeup of TRPA1. Many chemical agents, including formaldehyde, cinnamaldehyde (found in cinnamon), allyl isothiocyanate (found in mustard), and other reactive chemicals, can cause the TRPA1 channel to become activated. These irritants activate the channel, letting cations like calcium (Ca2+) into the cell and starting a signaling cascade that causes pain perception and inflammatory reactions[2].

1.2. TRPA1's function in lung diseases

Major worldwide health issues are lung disorders such as acute respiratory distress syndrome (ARDS), idiopathic pulmonary fibrosis,

asthma, and chronic obstructive pulmonary disease (COPD). These disorders have different etiologies and pathophysiology, but they all have inflammation, oxidative stress, and tissue remodeling in common, all of which gradually deteriorate lung function. The role of the TRPA1 channel as a crucial molecular mediator in these processes—particularly in reaction to irritants and inflammatory mediators inhaled—has recently come to light.

TRPA1 is expressed in a variety of lung cell types, such as neurons, smooth muscle, fibroblast, and airway epithelial cells. For example, activation of TRPA1 in the epithelium of the airways can result in increased mucus formation, bronchoconstriction, and the release of pro-inflammatory cytokines, all of which can contribute to symptoms that are common to asthma and COPD, such as inflammation, coughing, and shortness of breath. Moreover, TRPA1 contributes to nerve fiber sensitization, which might make patients with long-term lung illnesses feel more pain and suffering[5].

1.3. The function of lung cells in respiratory conditions

The several cell types that make up the lungs each have distinct functions in preserving lung function and reacting to disease stimuli. Important cell types include smooth muscle cells, endothelial cells, fibroblasts, macrophages, and alveolar epithelial cells (types I and II). The bulk of the alveolar surface is covered in type I alveolar epithelial cells, which are vital for gas exchange between blood and air. Apart from repairing the alveolar epithelium, type II alveolar epithelial cells also generate surfactant, a material that lowers surface tension and stops alveolar collapse[5].

By contracting and relaxing the airways, smooth muscle cells found in the walls of bronchi and bronchioles control airflow. The extracellular matrix is produced and maintained by fibroblasts, and fibrosis—a disease marked by excessive collagen deposition and

other matrix components—can result from the activation of these cells. This condition reduces lung flexibility and makes breathing more difficult. The blood arteries of the lungs are lined by endothelial cells, which are vital for controlling vascular tone and permeability as well as preserving adequate lung perfusion.

As important immune cells, macrophages play a critical role in identifying and getting rid of infections, but they may also cause tissue damage and inflammation in diseases like COPD and asthma. Lung disorders are determined by the complicated interactions between these cell types. TRPA1 channels are a potential therapeutic target in lung illnesses because they can control these processes when activated in different types of lung cells[5].

1.4. TRPA1 function knockout studies

They have been essential in expanding our knowledge of the TRPA1 channel. These studies entail deliberately deactivating certain genes to examine their functions. Mice carrying the TRPA1 knockout (KO) gene have proved useful in identifying the precise functions of this channel in a range of physiological and pathological conditions. Research with TRPA1 KO models has demonstrated that this channel is crucial for environmental responses since its absence decreases reactions to a variety of inhaled allergens and irritants.

For example, compared to wild-type mice, TRPA1 KO animals exhibit less airway inflammation, less bronchoconstriction, and decreased production of inflammatory mediators in models of allergen-induced asthma. These results provide compelling evidence that the TRPA1 channel plays a role in the pathophysiology of asthma and imply that targeted suppression of TRPA1 may be a useful treatment strategy. Furthermore, supporting the role of the TRPA1 channel in COPD, TRPA1 KO mice in COPD models show less lung tissue damage and a

decreased inflammatory response to toxic chemicals like tobacco smoke[12].

1.5. TRPA1channel inhibition through pharmacology

There is growing interest in creating pharmacological inhibitors of the TRPA1 channel as possible treatments for lung illnesses, building on data from KO trials. Numerous substances that selectively block the TRPA1 channel have been created and examined in preclinical research. Asthma and COPD may be treated with these inhibitors because of their encouraging effects, which include lowering airway hyperreactivity, reducing inflammation, and reducing pain sensitivity. Since the TRPA1 channel is also involved in normal physiological processes in other areas of the body, including as pain perception and blood pressure control, one of the primary problems in creating TRPA1 inhibitors is obtaining selectivity and minimizing side effects. Consequently, more investigation is required to maximize the selectivity of these inhibitors and learn more about the roles that TRPA1 plays in various tissues[9].

1.6. Future paths for research

The therapy of lung disorders and other medical issues may be greatly impacted by more study on the TRPA1 channel. Creating targeted TRPA1 channel inhibitors may provide patients with COPD, asthma, and other lung disorders new treatment choices, but it may also have wider uses in the management of illnesses linked to oxidative stress, chronic inflammation, and pain. Furthermore, a better comprehension of the molecular processes controlling the TRPA1 channel may result in more targeted therapy, which would lessen the adverse effects of the medications that are now in use.

Subsequent investigations might encompass an in-depth charting of TRPA1 channel manifestation in distinct cell kinds and tissues found in the lungs and additional organs. These investigations may reveal novel roles for the TRPA1 channel and shed light on how it functions in intricate physiological networks. Furthermore, studies examining how the TRPA1 channel interacts with other signaling molecules and pathways may help create combination treatments that address several facets of the pathophysiology of a given illness[36].

2. GOAL OF THE RESEARCH

This study's primary objective is to present a thorough analysis of the state of the art on the function of the TRPA1 channel in lung disorders, with an emphasis on COPD and asthma in particular. We will examine prospective therapeutic approaches that target the TRPA1 channel and critically evaluate the findings of KO studies that have illuminated the particular roles of this channel. We will investigate the potential future ramifications of these approaches in light of these studies, particularly with regard to the creation of novel medications and treatments for lung disorders.

Scientific framework and the requirement for critical review of literature

Over the past 10 years, the TRPA1 channel has been the subject of intense research; nonetheless, many questions remain unsolved despite tremendous advances. The role of the TRPA1 channel is interpreted differently in the literature, which is indicative of the molecular entity's complexity and multifunctionality. To determine current knowledge gaps, comprehend the intricacy of the TRPA1 channel's function, and progress the creation of novel treatment approaches, a careful review of the literature is required. This methodology facilitates the amalgamation of diverse research viewpoints and augments our comprehension of the function of the TRPA1 channel in both health and illness. In the end, a comprehensive critical study can serve as a basis for future studies focused on creating safe and efficient treatments that target the TRPA1 channel in a variety of clinical situations, with a focus on lung disorders in particular.

3. OVERVIEW OF THE TRPA CHANNEL FAMILY

The lungs are the body's entryways to the outside world and are crucial for gas exchange. The airways and the vasculature are easily accessible for medicines from both directions. Transient Receptor Potential (TRP) channels are implicated in stress-induced physiological responses as crucial components of signal transduction cascades and chemosensors, according to recent research. The TRP channels (TRPA1, TRPC6, TRPV1, and TRPV4) that are mostly expressed in non-neuronal lung tissues will be the subject of this work because of their connection to pathways linked to conditions such lung fibrosis, asthma, cystic fibrosis, chronic obstructive pulmonary disease (COPD), and edema production. Particular modulators of these channels that have recently been discovered and their promise as novel therapeutic alternatives as well as approaches for a causative therapy based on the mechanistic understanding of molecular events[1][2].

3.1. The TRPA channel family

A vast and varied family of ion channels known as transient receptor potential (TRP) channels is involved in many different physiological and sensory processes. The TRPA (Transient Receptor Potential Ankyrin) subfamily of TRP channels is distinct from the others since it plays a role in both sensing environmental stimuli and modulating the body's reaction to them. There are other members of the TRPA channel family, but TRPA1 has been investigated the most because of its important role in a number of pathophysiological diseases.
The cytoplasmic domains of the TRPA family are characterized by the presence of ankyrin repeats. The activation of channels and their interaction with other proteins depend on these ankyrin repeats. It is well known that TRPA channels are sensitive to a variety of

environmental stimuli, including as harmful substances, temperature fluctuations, and mechanical pressures. Because of their sensitivity, TRPA channels are important for a number of physiological and pathological processes, including cellular stress responses, inflammation, and pain perception[1].

3.2. Key members of the TRPA family

3.2.1. TRPA1

The most researched member of the TRPA subfamily, TRPA1 is activated by a variety of irritants, including as naturally occurring chemicals and toxins found in the environment. It contributes to the sense of pain, inflammation, and respiratory reactions and is expressed in sensory neurons, epithelial cells, and fibroblasts[2].

3.2.2. TRPA2

Another member of the TRPA subfamily, although one with less characterization than TRPA1. It is involved in several physiological processes and has some structural similarities with TRPA1. Less is known about its precise roles and involvement in illness, though[3].

3.2.3. TRPA3

Like TRPA1, TRPA3 is a channel in the TRPA family that is used to sense environmental cues. It is expressed in different tissues and aids in the identification of irritants as well as the control of physiological reactions[4].

3.2.4. TRPA4

A member of the TRPA family, TRPA4 is involved in a number of physiological and sensory processes. Although its function in illness is still being investigated, it is known that, in contrast to TRPA1, it is

triggered by distinct stimuli[5].

3.3. TRPA1 channel: overview and operation

TRPA1, or "ankyrin repeat domain-containing ion channel 1," is a homotetrameric ion channel made up of four subunits that are the same. Many ankyrin repeats are present in each subunit and are essential for channel activation and protein interaction [6]. Because of its exceptional sensitivity to a broad variety of chemical irritants, TRPA1 stands out among TRP channels.

3.4. Activation and sensitivity

A variety of harmful substances, including as tobacco smoke, formaldehyde, and reactive oxygen species, can activate TRPA1. Additionally, it is susceptible to substances found in nature, such garlic, cinnamon, and mustard oil [7]. When TRPA1 is activated, cations such as calcium (Ca2+) and sodium (Na+) can enter the system and cause a variety of cellular reactions, including the release of inflammatory mediators and the activation of pain pathways [8].

3.5. Function in pain and inflammation

TRPA1 is an essential mediator of both pain and inflammation. It is expressed in sensory neurons and has a role in the sense of discomfort and pain. Pro-inflammatory cytokines and other mediators are released when irritants activate TRPA1, exacerbating pain and inflammation[9]. Because of this, TRPA1 may be used as a therapeutic target to treat inflammatory and pain-related disorders.

3.6. Function in respiratory illnesses

TRPA1 is expressed in smooth muscle cells, fibroblasts, and airway epithelial cells in the setting of respiratory illnesses. higher mucus production, bronchoconstriction, and higher susceptibility to irritants can result from its activation in these cells. TRPA1 leads to airway inflammation and hyperreactivity in diseases such asthma and chronic obstructive pulmonary disease (COPD)[10]. Research has demonstrated that TRPA1 mediates the effects of inhaled irritants and exacerbates symptoms like wheezing and dyspnea, which contributes to the pathogenesis of these diseases[11].

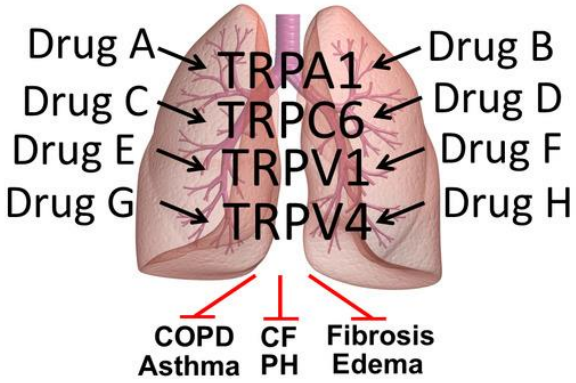

Figure 1. Graphical abstract of the text above (3.6)

4. OVERVIEW OF LUNG CELLS AND THEIR FUNCTIONS

4.1. Introduction to lung cells

The vital organs that facilitate gas exchange—allowing carbon dioxide to leave the body and oxygen to enter the bloodstream—are the lungs. The lungs' highly specialized structure is made up of several cell types that cooperate to support respiratory function. These cells can be roughly classified as immune cells, fibroblasts, endothelial cells, and epithelial cells. Each of these cell types has a unique purpose in preserving lung health and function.

4.2. Types of lung cells

4.2.1. Epithelial cells

The airways and alveoli are lined by epithelial cells, which provide a barrier between the external environment and the lungs' interior components. They are further classified into various subtypes:

4.2.2. Type I alveolar cells (pneumocytes)

These cells comprise approximately 95% of the alveolar surface and are principally responsible for gas exchange. They have a thin, flattened shape that allows for quick gas diffusion over the alveolar membrane[12].

4.2.3. Type II alveolar cells (pneumocytes)

These cells are cuboidal in form and release pulmonary surfactant, a chemical that lowers surface tension in the alveoli, avoiding collapse during exhale. Type II cells also function as progenitor cells for Type I alveolar cells, which is critical for alveolar regeneration following injury[13].

Airway epithelial cells line the trachea, bronchi, and bronchioles. They act as the initial line of defense against inhaled infections, particles, and poisons. Airway epithelial cells create mucus and have cilia that work together to capture and remove foreign particles from the lungs via the mucociliary escalator [14].

4.3. Endothelial cells

Endothelial cells line the blood arteries in the lungs and are necessary for the pulmonary vasculature to function properly. These cells govern the exchange of chemicals between the blood and surrounding tissues, maintain blood pressure in the lungs, and participate in inflammatory reactions. Endothelial dysfunction is linked to a variety of lung conditions, including pulmonary hypertension and acute lung injury[15].

4.4. Immune cells

The lungs contain a variety of immune cells that guard against infections and assist maintain tissue homeostasis.

4.4.1. Alveolar macrophages

These immune cells live within the alveoli. They phagocytose (engulf and consume) pathogens, dead cells, and detritus. Alveolar macrophages also help to regulate inflammatory responses and maintain tolerance to non-harmful antigens [16].

4.4.2. Dendritic cells

Dendriticc cells are antigen-presenting cells that collect and process foreign antigens, triggering immunological responses by activating T cells. They are essential for the adaptive immune response in the lungs[17].

4.4.3. Lymphocytes

Adaptive immunity is mediated by lymphocytes, which include T and B cells. T lymphocytes provide cell-mediated immunity, whereas B cells create antibodies that target particular pathogens[18].

4.5. Fibroblasts

Fibroblasts are connective tissue cells that create the extracellular matrix (ECM), which provides structural support for the lungs. In addition to producing ECM, fibroblasts aid in wound healing and tissue restoration. However, in chronic lung illnesses such pulmonary fibrosis, fibroblasts can become hyperactive, resulting in excessive ECM deposition and scarring, impairing lung function[19].

4.6. Lung cell involvement in disease

The numerous cell types in the lungs have diverse roles in both health and sickness. Disruption in the normal operation of these cells can cause a variety of respiratory conditions:

4.6.1. Chronic obstructive pulmonary disorder (COPD)

COPD is defined by persistent inflammation, airway remodeling, and alveolar damage. In COPD, airway epithelial cells and immune cells, mainly macrophages and neutrophils, contribute to the chronic inflammatory state. Endothelial cell dysfunction and fibroblast activation contribute to the development of pulmonary hypertension and emphysema, respectively[20].

4.6.2. Asthma

Asthma is a chronic inflammatory illness of the airways that is distinguished by bronchoconstriction, mucus hypersecretion, and hyperresponsiveness. Airway epithelial cells and immune cells,

notably eosinophils, mast cells, and T lymphocytes, play critical roles in the pathogenesis of asthma. The interaction of epithelial cells with immune cells results in the production of cytokines and chemokines, which promote inflammation and airway remodeling [21].

4.6.3. Pulmonary fibrosis

Pulmonary fibrosis is a progressive lung illness characterized by excessive ECM deposition, which causes scarring and hardening of the lung tissues. Fibroblasts play an important part in this process by activating and converting into myofibroblasts, which create huge quantities of collagen and other ECM components. Excessive ECM synthesis causes thickening of the alveolar walls and poor gas exchange[22].

4.6.4. Lung cancer

Lung cancer is caused by the uncontrolled multiplication of cells inside the lungs, primarily epithelial cells. The two most common types of lung cancer are non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). Malignant transformation in airway epithelial cells can be caused by genetic alterations, chemical exposure (such as tobacco smoke), or chronic inflammation. The tumor microenvironment, which comprises immune cells and fibroblasts, also has an impact on tumor development and metastasis [23].

4.7. Interaction between lung cells with TRPA1 channel

As previously mentioned, the TRPA1 channel is expressed in a variety of lung tissues, including epithelial cells, smooth muscle cells, and fibroblasts. The activation of TRPA1 in these cells has substantial ramifications for respiratory health:

Environmental irritants activate TRPA1 in airway epithelial cells, resulting in the release of pro-inflammatory cytokines and the

formation of mucus. This contributes to the pathogenesis of asthma and COPD, which are characterized by persistent inflammation and mucus hypersecretion [24].

TRPA1 activation in fibroblasts may contribute to the development of pulmonary fibrosis by increasing fibroblast proliferation and ECM synthesis. Targeting TRPA1 in fibroblasts might be a new therapeutic method for preventing or slowing the evolution of fibrosis in illnesses such idiopathic pulmonary fibrosis[25].

4.7.1. TRPA1 and immune cells

Although TRPA1 is not normally expressed in immune cells, its activation in epithelial cells and fibroblasts can indirectly influence immune responses by altering cytokine and chemokine release, modulating immune cell recruitment and activation in the lungs[26].

2008, *38*, 1548–1558. [Google Scholar] [CrossRef] [PubMed]

		RPC6	TRPV1	TRPV4
Bronchial epi.	−/+[44] [1], [43] [3]	+[123] [2]	+[124] [3]	+[92] [2]
Airway SMC	−/+[44] [1]	+[123] [2]	+[70] [3]	+[104] [2]
AT1 cells	−/+[44] [1]	?	?	?
AT2 cells	−/+[44] [4]	?	?	?
Alveolar MP	?	+[22] [4]	?	+[125] [5]
Endothelium	−/+[44] [1]	+[19] [4]	+[126] [2]	+[126] [2]
PASMC	−/+[44] [1]	+[18] [2]	+[127] [4]	+[127] [2]
Neutrophils	?	+[23] [3]	?	+[23] [2]
Fibroblasts	−/+[44] [1]	−/+[21] [2]	?	+[128] [4]
Myofibroblasts	?	+[21] [4]	?	+[128] [4]

−/+, very low expression, +, expression, ++, high expression, ?, not tested. References in brackets []. Detection by: [1] labeling of specific mRNA (nanostring® technology); [2] amplification of specific mRNA by quantitative reverse transcription (qRT)-PCR; [3] imunohistochemistry, [4] labeling protein by specific antibodies in a Western Blot, [5] functional assays.

Table 1. TRP expression patterns in lung cells.

5. LUNG DESIASES AND TRPA CHANNELS' POTENTIONAL ROLE IN TREATMENT

5.1. Overview of lung diseases

Lung disorders are a major worldwide health burden, affecting millions of people and resulting in significant morbidity and mortality. The most prevalent and serious lung illnesses include COPD, asthma, pulmonary fibrosis, and lung cancer. Each of these disorders includes complicated pathophysiological processes, which are frequently triggered by chronic inflammation, tissue remodeling, and cellular dysfunction. Smoking is a significant risk factor for several lung disorders, including COPD and lung cancer. Recent research indicates that Transient Receptor possible Ankyrin 1 (TRPA1) and other TRPA channels may play key roles in many disorders, providing possible therapeutic targets.

5.1.1. Chronic obstructive pulmonary disorder (COPD)

COPD is a progressive lung disease marked by airflow restriction that is not completely reversible. persistent inflammation in the airways and alveoli causes symptoms including persistent cough, sputum production, and shortness of breath. COPD is essentially defined as chronic bronchitis and emphysema, both of which are significantly associated to tobacco smoke exposure and other environmental contaminants [12].

The TRPA1 channel, which detects reactive chemical species and environmental irritants, is linked in the worsening of COPD symptoms. Smoking, as a significant source of these irritants, can activate TRPA1 in the airways, resulting in the release of pro-inflammatory cytokines and increased mucus production, which contribute to airway blockage and hyperresponsiveness [13]. TRPA1 inhibition has been recommended as a treatment method for reducing inflammation and improving lung function in COPD patients [14].

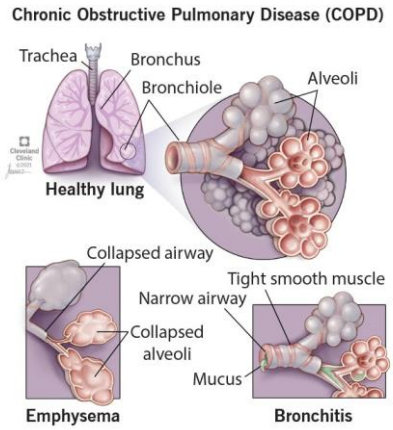

Figure 2. Healthy vs. COPD influenced lungs.

5.2. Asthma

Asthma is a chronic inflammatory illness of the airways that causes wheezing, shortness of breath, chest tightness, and coughing. Asthma inflammation causes hyperresponsive airways and reversible airway blockage. TRPA1 is found in airway epithelial cells and sensory neurons, where it reacts to environmental irritants such pollution, allergens, and smoking (15).

Smoking can worsen asthma symptoms by activating TRPA1 in these cells, which causes bronchoconstriction, mucus formation, and the release of pro-inflammatory mediators. Targeting TRPA1 in asthma treatment may lower airway inflammation and hyperresponsiveness. TRPA1 antagonists, for example, may reduce symptoms in people with allergic asthma by inhibiting channel activity in response to irritants [16].

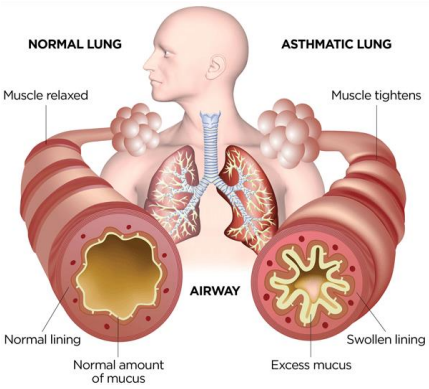

Figure 3. Normal lungs vs. asthmatic lung.

5.3. Pulmonary fibrosis

Pulmonary fibrosis is a chronic and progressive lung illness characterized by excessive deposition of extracellular matrix proteins, which causes scarring and stiffness of the lung tissue. This leads to poor gas exchange and diminished lung compliance. The illness is commonly idiopathic, although it can also be caused by environmental factors such as smoking, certain drugs, and chronic inflammatory diseases [17].

TRPA1 is expressed by lung fibroblasts, which are principally responsible for extracellular matrix formation. In pulmonary fibrosis, TRPA1 activation has been associated to fibroblast proliferation and excessive collagen synthesis, which contribute to tissue scarring. Smoking, which activates TRPA1, may thereby contribute to the advancement of fibrosis. Inhibiting TRPA1 may diminish fibroblast activation and halt the evolution of fibrosis [18].

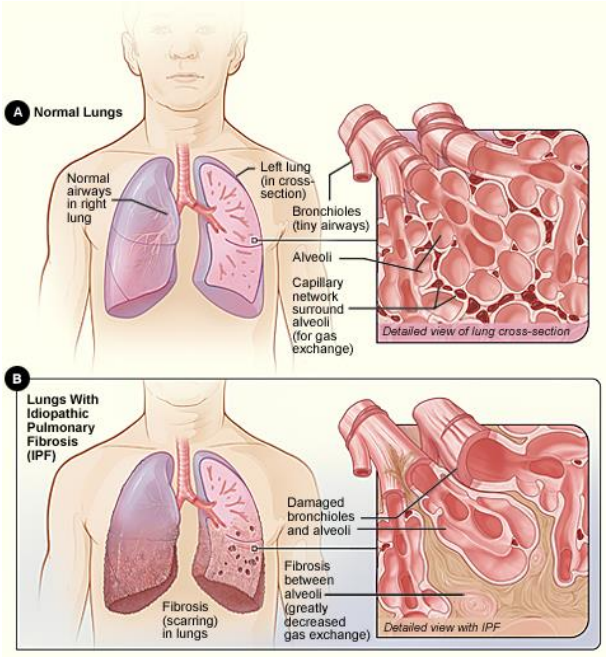

Figure 4. Normal lungs vs. lungs with IPF.

5.4.Lung cancer

Lung cancer is one of the most common causes of cancer-related mortality globally. The condition is characterized by uncontrolled cell proliferation within the lungs, which frequently originates from the epithelial cells that border the airways. Smoking is the leading cause of lung cancer, contributing to DNA damage and cellular changes that lead to malignancy [19].

Recent research suggests that TRPA1 may be involved in the development of lung cancer. TRPA1 is expressed in lung cancer cells, and activation can stimulate cell proliferation, migration, and invasion. Furthermore, TRPA1 activation in the tumor microenvironment may contribute to cancer-associated inflammation, which promotes tumor development and metastasis. Smoking, being a powerful TRPA1 activator, may increase the course of lung cancer. Targeting TRPA1 may be a potential technique in lung cancer therapy, either alone or in conjunction with other therapies [20].

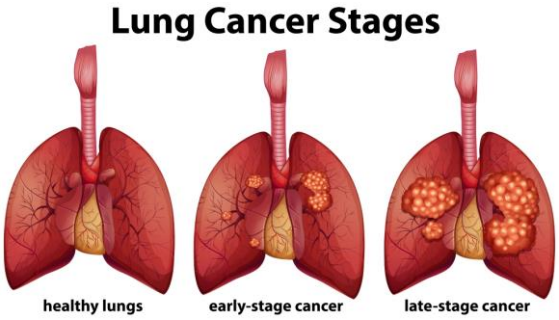

Figure 5. Lung cancer through stages.

6. CONNECTION BETWEEN TRPA CHANNELS. KO STUDIES AND PULMONARY DISEASES

6.1. Introduction to knockout experiments

Knockout (KO) experiments entail deliberately deleting or inactivating certain genes inside an organism in order to examine their function. Researchers can infer the involvement of a gene in normal biological processes and disease states by analyzing the physiological and pathological changes that occur when it is absent. KO models, particularly in mice, have become essential tools in biomedical research for clarifying the functions of numerous genes, including those that code for ion channels such as the Transient Receptor Potential Ankyrin 1 (TRPA1) channel [21].

6.2. TRPA1 knockout studies in asthma

Asthma is a chronic inflammatory condition characterized by increased airway reactivity, mucus production, and bronchoconstriction. TRPA1 is expressed in airway epithelial cells and sensory neurons, where it detects environmental irritants such as pollution, allergens, and cigarette smoke. Studies on TRPA1 KO mice have shown that allergen exposure results in a considerable decrease in airway hyperreactivity and mucus production. In a model of ovalbumin-induced asthma, TRPA1 KO mice showed less airway

inflammation and hyperresponsiveness than wild-type mice [22]. These findings indicate that TRPA1 plays an important role in modulating the airway inflammation and hyperreactivity associated with asthma, making it a viable therapeutic target.

6.3. TRPA1 and COPD: findings from knockout studies

TRPA1 has also been linked to chronic obstructive pulmonary disease (COPD), a serious respiratory illness. COPD is characterized by chronic inflammation, airway obstruction, and emphysema, which are frequently aggravated by cigarette smoke and environmental contaminants. TRPA1 knockout experiments have revealed important insights into how this channel contributes to COPD pathogenesis. TRPA1 KO animals demonstrated much less airway inflammation, decreased mucus formation, and lower levels of oxidative stress in cigarette smoke-induced COPD mouse models than wild-type mice [23]. These findings show that cigarette smoke and other irritants activate TRPA1, which plays an important role in the inflammatory and oxidative processes that promote COPD development.

6.4. Pulmonary fibrosis and TRPA1 knockout studies

Pulmonary fibrosis is defined by the excessive deposition of extracellular matrix proteins, which causes scarring and hardening of lung tissue. The disease might be idiopathic or caused by chronic inflammatory diseases or environmental exposures, such as cigarette smoke. TRPA1 has been demonstrated to be involved in the stimulation of fibroblasts, which produce collagen in response to oxidative stress and other stimuli. KO experiments have shown that TRPA1-deficient animals are less likely to develop lung fibrosis in response to fibrotic drugs such as bleomycin. TRPA1 knockout animals showed lower fibroblast proliferation and collagen deposition

in both models, indicating that TRPA1 plays a role in the fibrotic processes that define pulmonary fibrosis [24]. These results emphasize the potential of TRPA1 inhibitors as therapeutic agents in treating pulmonary fibrosis.

6.5. Lung Cancer, TRPA1, and knockout studies

One of the most prevalent and lethal types of cancer in the world is lung cancer, especially non-small cell lung cancer (NSCLC). Numerous lung cancer cell lines contain TRPA1, which has been linked to enhancing cell invasion, migration, and proliferation. Research using KO mice as lung cancer models has demonstrated that a lack of TRPA1 results in decreased tumor development and metastasis. For example, compared to wild-type mice, TRPA1 KO animals in models of chemically induced lung cancer generated fewer and smaller tumors [25]. This implies that TRPA1 could be a target for lung cancer treatment and might play a role in carcinogenesis.

6.6. TRPA1, smoking, and pulmonary disease knockout research

Numerous respiratory conditions, such as COPD, asthma, pulmonary fibrosis, and lung cancer, are known to be exacerbated by smoking. The activation of TRPA1 channels in response to the many reactive compounds included in cigarette smoke plays a role in mediating the detrimental consequences of smoking. The KO experiments have played a crucial role in illustrating how the lack of TRPA1 might lessen the harmful effects of smoking on the lungs. For instance, compared to wild-type mice, TRPA1 KO animals exposed to cigarette smoke had decreased oxidative stress, decreased airway inflammation, and a lower incidence of emphysema [26]. These results support the hypothesis that TRPA1 mediates the detrimental effects of smoking on the respiratory system.

7. TRPA1 CHANNEL INHIBITION THROUGH PHARMACY

Mechanisms of Inhibition of the TRPA1 Channel
Several methods, such as competitive antagonism, allosteric modulation, and direct blocking of the channel's pore, can lead to the pharmacological suppression of TRPA1 channels. Every technique seeks to avoid or lessen the pathogenic consequences of TRPA1 activation.

7.1. Competitive antagonists

Substances that attach directly to the active site of the TRPA1 channel, preventing endogenous activators from interacting with one another. For instance, it is known that by attaching to TRPA1's ligand-binding domain, HC-030031 and A-967079 competitively inhibit the protein [27]

7.2. Allosteric Modulation

By binding to locations other than the main active site, allosteric modulators alter the activity and conformation of the channel. The channel's responsiveness to stimuli can be either enhanced or inhibited by these modulators. A more sophisticated method of regulation is offered by allosteric inhibition of TRPA1, which affects channel activity without directly competing with endogenous activators [28].

7.3. Direct Blockade

This technique uses substances to obstruct TRPA1's ion-conducting pore, stopping ion flow. Targeting the pore of the channel, small

compounds as GRC 17536 have been demonstrated to directly block TRPA1 [29].

7.3.1. Pharmacological Blockade and Pain Control

In particular, TRPA1 channels are important for the experience of pain in chronic pain and inflammation. Treatment for these disorders appears to be improved by inhibitors that target TRPA1. The well-known TRPA1 antagonist HC-030031 has been effective in lowering pain responses in animal models of neuropathic and inflammatory pain [30]. These findings imply that by reducing the sensory and inflammatory reactions mediated by this channel, TRPA1 inhibitors may offer relief from chronic pain.

7.4. Inhibition of TRPA1 in Respiratory Conditions

Inhibiting TRPA1 may potentially be used to treat respiratory conditions such pulmonary fibrosis, COPD, and asthma. In animal models of these disorders, it has been demonstrated that inhibition of TRPA1 reduces mucus formation, hyperreactivity, and airway inflammation. For instance, in mouse models, it has been observed that the TRPA1 inhibitor A-967079 reduces airway inflammation and the intensity of asthma symptoms [31]. In a similar vein, TRPA1 inhibitors have reduced inflammation and airway blockage caused by cigarette smoke exposure in COPD models [32].

TRPA1 suppression is beneficial for pulmonary fibrosis, which is characterized by excessive formation of extracellular matrix and tissue scarring. In pulmonary fibrosis models, studies employing TRPA1 inhibitors have demonstrated decreased fibroblast proliferation and collagen deposition. For example, it has been demonstrated that in response to fibrotic stimuli, the TRPA1 antagonist GRC 17536 reduces the synthesis of collagen and

fibroblast activation [33]. These findings imply the possibility of using TRPA1 inhibitors to control or impede the advancement of pulmonary fibrosis.

8. FUTURE RESEARCH AND DEVELOPMENT OF CURES THROUGH TRPA1 CHANNELS

8.1. Extending TRPA1 inhibition's therapeutic targets for pain management and neuropathic pain

Upcoming studies will probably concentrate on creating more precisely tailored TRPA1 inhibitors that will target pain pathways without interfering with regular sensory processes. Although preclinical research on some of the current inhibitors, such as HC-030031 and A-967079, has showed promise, the objective is to increase these drugs' effectiveness and minimize their adverse effects [36][37]. New types of TRPA1 antagonists are being investigated by researchers in hopes of improving pain treatment for neuropathic pain and fibromyalgia.

8.2. Respiratory diseases

Current research in this field is aimed at creating TRPA1 inhibitors that would improve the management of airway illnesses such as asthma and chronic obstructive pulmonary disease (COPD). Novel approaches to medication administration, including inhaled formulations, are being investigated to directly target TRPA1 channels in the respiratory tract [38]. Furthermore, research is looking at how environmental contaminants and allergens might exacerbate respiratory diseases, and how TRPA1 may play a part in this.

8.3. Pulmonary fibrosis

Because of its progressive nature and the dearth of effective therapies, pulmonary fibrosis poses a considerable problem. The goal of research is to identify the precise processes via which TRPA1 causes fibrosis, particularly its involvement in collagen deposition and

fibroblast activation [39]. The goal of future research is to create TRPA1 inhibitors that precisely target fibrotic processes in order to slow or perhaps stop the disease's growth.

8.4. Novel approaches to the development of TRPA1 selective inhibitors

One of the key priorities is still developing highly selective TRPA1 inhibitors. Issues with off-target effects and interactions with other ion channels are common problems for current inhibitors [40]. Novel strategies, such structure-based drug design and high-throughput screening, are being utilized to find novel compounds that modify TRPA1 alone, influencing other TRP channels in an exclusive manner.

8.5. Combination therapies

The effectiveness of therapy may be increased by combining TRPA1 inhibitors with other medicinal substances. For instance, combining TRPA1 antagonists with analgesics or anti-inflammatory medications may offer a more thorough method of treating respiratory disorders and chronic discomfort. In order to maximize treatment outcomes, research is investigating the synergistic effects of such combinations [41].

8.6. Proteomics and genomics developments

They are opening the door for customized medical techniques. Customizing TRPA1-targeted treatments according to each patient's unique genetic and molecular profile may be the focus of future study. By avoiding side effects and identifying patients who are most likely to benefit from TRPA1 inhibition, this tailored strategy may be able to aid [42].

8.7. Addressing challenges and future directions tolerance and safety

It is essential to guarantee the safety and tolerability of TRPA1 inhibitors. Clinical studies need to keep a close eye on any possible adverse effects as well as any long-term effects on regular physiological processes. To avoid unexpected outcomes, research must address the trade-off between preserving overall channel function and effectively modulating TRPA1 [43].

8.8. Mechanistic understanding

A deeper understanding of TRPA1's role in various disease mechanisms is essential. Future research should focus on elucidating the precise pathways through which TRPA1 contributes to disease progression and how its inhibition can modify these pathways. This knowledge will inform the design of more effective and targeted therapies [44].

8.9. Regulatory and clinical trials

Advancing TRPA1 inhibitors from preclinical studies to clinical practice involves navigating regulatory challenges and conducting rigorous clinical trials. Collaboration between researchers, clinicians, and regulatory bodies will be necessary to ensure that new therapies are evaluated comprehensively and meet safety and efficacy standards [44].

Conclusion

8.10. Future studies and developments pertaining to TRPA1 channels

They have great potential to progress the therapeutic choices available for pulmonary fibrosis, respiratory disorders, and chronic pain. To fully use TRPA1 regulation for therapeutic purposes, researchers are concentrating on combination medicines, tailored methods, and specific inhibitors. Improving patient outcomes and integrating these advances into clinical practice will depend on addressing issues with safety, mechanistic knowledge, and clinical trials.

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10.CURRICULUM VITAE

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RADNO ISKUSTVO

20/8/2023 – 7/9/2023 Slavonski Brod, Hrvatska

OBAVEZNA STRUČNA PRAKSA VODOVOD; ODJEL ZA KONTROLU ČISTOĆE VODE

Određivanje koncentracija ionskih vrsta prisutnih u otopini,upotreba volumetrijskih te spektrometrijskih metoda u vidu kontrole čistoće vode.

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Razina Europskoga kvalifikacijskog okvira razina 1 EKO-a

JEZIČNE VJEŠTINE

Materinski jezik/jezici: **HRVATSKI**

Drugi jezici:

	RAZUMIJEVANJE		GOVOR		PISANJE
	Slušanje	Čitanje	Govorna produkcija	Govorna interakcija	
ENGLESKI	C1	C1	C1	C1	C1

Razine: A1 i A2: temeljni korisnik; B1 i B2: samostalni korisnik; C1 i C2: iskusni korisnik

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