Famutimi *et al.*
Future Journal of Pharmaceutical Sciences (2024) 10:126
https://doi.org/10.1186/s43094-024-00709-y

Future Journal of Pharmaceutical Sciences

REVIEW

Open Access

Trypsin, chymotrypsin and elastase in health and disease

Oladoyin Grace Famutimi[1*], Victor Gbolahan Adebiyi[1], Bukola Grace Akinmolu[1], Omoniyi Vincent Dada[1] and Isaac Olusanjo Adewale[1]

Abstract

Background Serine proteases represent over 1% of all proteins in humans. This family of proteins is found on cell surfaces, subcellular organelles like lysosomes or mitochondria, within the nucleus and the protoplasm.

Main body of the abstract Among them, trypsin, chymotrypsin and elastase have aroused great interest because of their numerous functions in pathophysiological processes. Altered expression of these enzymes in experimental animal models and humans has been related to various pathologies, like developmental defects, metabolic dysfunctions, cancer, peripheral vascular diseases and infectious diseases. Trypsin and chymotrypsin-like proteases activate, or less oftentimes inactivate, numerous substrates, together with growth factors, receptors, adhesion molecules, angiogenic factors and metalloproteases. Among these substrates, a number of them are key factors in cancer progression, metastasis and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) disease. Elastin-degrading enzyme- elastase, slowly damages elastin over the lifetime of an organism. The physiological processes triggered by elastase leads to the progression of different conditions such as cancer, metabolic syndrome, pulmonary emphysema, atherosclerosis, and chronic obstructive pulmonary disease. These serine proteases are currently considered to be targets for the development of new potent therapeutics.

Short conclusion The cumulative knowledge that outlined the physiological functions and pathological implications of these proteases and the proposed strategies to regulate a number of their activities and their targeting for therapeutic application and validation in selected disease states are highlighted. These should enhance our appreciation of their roles in aetiology of some diseases as well as the chemotherapeutic benefits of their inhibition or modulation.

Keywords Serine proteases, Trypsin, Chymotrypsin, Elastase, Pathophysiology, Therapeutic target

Background

The diversity of proteins and peptides encoded by an organism genome is increased by multiple processes. Among these, are the post-translational modifications (PTMs) of primary protein products. Most PTMs influence the structure and function of these modified products, and are introduced through specific enzymes into target peptides or proteins [1]. With over 300 PTMs currently known, some modifications are known to be reversible while some are irreversible. Reversible modifications form a feedback mechanism that ensures the normal functioning of the cell under physiological conditions. They include: serine, threonine or tyrosine phosphorylation and S-nitrosylation [2].

Irreversible modifications are due to the nature of the biological function enabled by the modification. The most common irreversible modification is the cleavage of peptide substrate at specific sites via limited proteolysis to generate multiple products. The released products can be covalently modified further to shape the ultimate

*Correspondence:
Oladoyin Grace Famutimi
famutimioladoyin@gmail.com
[1] Department of Biochemistry and Molecular Biology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria

form of bioactive entities. Approximately 600 distinct proteases were revealed in the analysis of human and mouse genomes. These proteases are grouped into five major classes based on the interactions between their peptide substrate and the nucleophilic amino acid in the active site of the protease [2]. Among these are the serine, cysteine and threonine proteases that use their own respective amino acid to hydrolyze peptide bonds, while aspartic acid and metallo-proteases use water as nucleophile to break peptide bonds.

Serine proteases are the most common and diverse class of enzymes. They make up about one percent of all proteins present in humans [3]. Serine proteases are classified on the basis of their substrate specificity into several types including trypsin-like, subtilisin-like, chymotrypsin fold, elastase-like, etc. [4]. Also, these proteases are categorized into numerous clans on the basis of their catalytic site topology. Serine protease inhibitors, also known as serpins, control or regulate the activity of these proteases [5, 6]. Serpins are specific in nature, i.e., they inhibit enzyme activities by forming complexes with specific serine proteases.

Serine proteases possess a wide range of potential applications due to their involvement in diverse biological processes and their pivotal role in disease progression. These enzymes are integral to various physiological functions, such as immunity, regulation of development, metabolism, blood coagulation, digestion, and fertilization [7]. However, their dysregulation can contribute significantly to disease states, highlighting their potential as therapeutic targets.

For example, trypsin and chymotrypsin have been extensively studied for their roles in cancer. These serine proteases are not only involved in normal physiological processes but also contribute to the pathology of several cancers. In cancer biology, trypsin and chymotrypsin facilitate tumor invasion, proliferation, and metastasis by degrading extracellular matrix components and activating other proteases, growth factors, and signaling molecules. Overexpression of trypsin in cancers such as colorectal cancer has been associated with poor prognosis and reduced disease-free survival, suggesting that trypsin may serve as a biomarker for aggressive tumor phenotypes [8, 9]. Similarly, chymotrypsin-like proteases play a role in tumorigenesis by modulating the tumor microenvironment, promoting angiogenesis, and enhancing the metastatic potential of cancer cells.

Beyond their roles in cancer, elastase is another serine protease that plays a significant role in disease progression, particularly in chronic inflammatory conditions. Elastase is involved in tissue remodeling and immune responses but can cause extensive tissue damage when its activity is not properly regulated. For example, neutrophil elastase, which is released during inflammation, contributes to the destruction of alveolar tissue in the lungs, exacerbating conditions such as chronic obstructive pulmonary disease (COPD) and emphysema [10]. Elastase also plays a role in cardiovascular diseases such as atherosclerosis, where it degrades elastic fibers in blood vessels, leading to vessel wall stiffening and plaque formation. The pathological activities of elastase make it a prime target for therapeutic intervention, and elastase inhibitors are being investigated for their potential to mitigate tissue damage in chronic inflammatory diseases.

Interestingly, serine proteases are also found as toxins in the venoms of various organisms, including snakes, jellyfish, and other venomous species. These venom-derived serine proteases target the host's coagulation pathways, leading to severe consequences such as hypovolemia, systemic bleeding, and hemodynamic shock [6, 11]. By hydrolyzing clotting factors and inhibiting clot formation, these venom proteases disrupt normal blood flow and cause life-threatening conditions. Understanding the mechanisms of action of venom-derived serine proteases has not only provided insights into venom toxicity but also spurred the development of antivenoms and therapeutics to counteract venom-induced coagulopathies.

Serine proteases are synthesized as inactive precursors known as zymogens, which are activated through proteolytic cleavage. This activation process is tightly regulated to prevent uncontrolled protease activity that could lead to tissue damage. Serine proteases are produced in various cell types, including blood monocytes, mast cells, and neutrophils, where they play roles in immune responses and tissue remodeling [12]. For instance, neutrophil elastase acts as a potent mucus stimulant and plays a role in the pathogenesis of lung diseases by promoting inflammation and tissue damage.

Given their critical roles in both physiological and pathological processes, serine proteases especially trypsin, chymotrypsin, and elastase, represent promising targets for therapeutic intervention. Inhibitors of these enzymes are being explored as potential treatments for a wide range of diseases, including cancer, inflammatory conditions, and venom-induced pathologies. By targeting the dysregulated activity of these proteases, it may be possible to develop therapies that not only halt disease progression but also restore normal physiological function. This review focuses on the physiological and pathological roles of trypsin, chymotrypsin, and elastase, with an emphasis on how their inhibition may offer considerable prospects for therapeutic applications.

Trypsin

Trypsin (EC 3.4.21.4) belongs to pancreatic serine proteases from the Protease of mixed nucleophile,

Superfamily A (PA clan). The enzyme is located in the digestive system. It is excreted in an inactive proenzyme form trypsinogen by the pancreas. Trypsinogen later enters the small intestine where it is converted to trypsin upon digestion via the bile duct. Enterokinase is an enzyme that activates trypsinogen to trypsin in a process known as proteolytic cleavage. Trypsin functions by hydrolyses of protein. They hydrolyze the amino acids lysine or arginine mainly at the carboxyl end [13].

Role of trypsin in diseases

Tumor progression Trypsin is involved in the progression of different types of cancer. It is involved in the process of angiogenesis/proliferation, invasion and metastasis in the entire carcinogenic processes [14]. Although trypsin is expressed in different types of cancer, it is more common in ovarian and colorectal cancer among others. The aggressiveness of tumors is affected by the overexpression of trypsin. Trypsin does not just function intracellularly to affect the carcinogenic process; its function has also been studied extracellularly in both in vivo and in vitro models [9]. It degrades extracellular matrix (ECM) components and affects the activation cascades of other proteases like Matrix Metalloproteinases (MMPs) which have been found to promote the invasion of tumors [9, 15].

The mechanism by which trypsin induces invasion and metastasis is divided into three phases: (a) Trypsin attacks extracellular proteins and degrades them (b) Trypsin activates other proteolytic cascades, such as the MMPs (c) Signal molecules such as Protease-activated Receptor-2 (PAR2) are activated [14]. The activation of MMPs by trypsin has been found to play a significant role in the progression of cancer as the presence of trypsin acting in concert with overexpressed MMPs (MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-12, MMP-13, MMP-14) contribute to the process of proliferation, progression and invasion [14, 16].

For example, in colorectal cancer adenoma, a benign, non-cancerous tumor is converted to carcinoma (a malignant lesion) and spread, systemically through metastasis and it has been noted that overexpression of some MMPs has a role to play in this process [14, 17]. Protease-activated Receptor-2 (PAR-2) is a G-protein-coupled receptor (GPCR) primarily activated by trypsin-like serine proteases, for signal transduction pathways. It plays vital roles in body physiology as well as in aetiology of some diseases including cancer, obesity, gastrointestinal, respiratory, metabolic and central nervous system disorders [18]. Trypsin is the most effective activator of PAR-2 and PAR-2 can also activate trypsin and this leads to an autocrine loop. PAR-2 has been observed to promote metastasis and motility of cancer cells (Fig. 1) [14, 19].

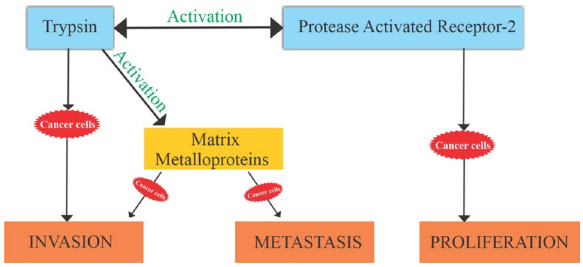

Fig. 1 The Interaction of Trypsin with Proteinase Activated Receptor-2 in Cancer Aetiology. Trypsin can be activated by Protease Receptor-2 (PAR-2) and PAR-2 can activate trypsin. The two have been implicated in cancer progression and metastasis [14]

Despite the role of trypsin in the progression of cancer, its actual function/action in ovarian cancer is not well understood [9]. In OV90 ovarian cancer cells, PARs were expressed in clinical ovarian cancer tissues. PAR-2 may represent a new therapeutic target for potentially treating ovarian cancer metastasis [18].

Cystic fibrosis Cystic fibrosis is a muco-obstructive lung disease that occurs due to a genetic disorder. This disorder causes an adverse effect on the transport of ions in the exocrine glands. The ions transported are insufficient and this results in the build up of thick secretions in vital organs such as lungs, pancreas and intestines [20].

Trypsin activates other proenzymes like chymotrypsinogen and a deficiency of trypsin and other pancreatic enzymes have been found to be closely linked to a neonatal condition known as meconium ileus. This disease is associated with a blockage in the ileum of the small intestine which is caused by abnormally thick and sticky meconium. Meconium ileus is one of the earliest manifestations of cystic fibrosis [21].

Trypsin-like proteases (TLPs) also play a role in muco-obstructive lung diseases. They activate the epithelial sodium channel (ENaC) that causes dehydration and mucus secretion in the airways of the lungs. Studies show that high level of activity of TLPs results in poorer lung function which is crucial in the identification of biomarkers and therapeutic interventions. Prostasin and matriptase, in addition to a number of other soluble epithelial-derived TLPs are found in the airways of the lungs due to increased activity of TLPs and this can aid hyperactivation of sodium epithelial channel and dehydration of airways which are major initiating factors in the onset of cystic fibrosis (Fig. 2) [22].

Pancreatitis The major causative event that leads to the onset of pancreatitis is the autoactivation of digestive enzymes in the pancreas. Under normal conditions,

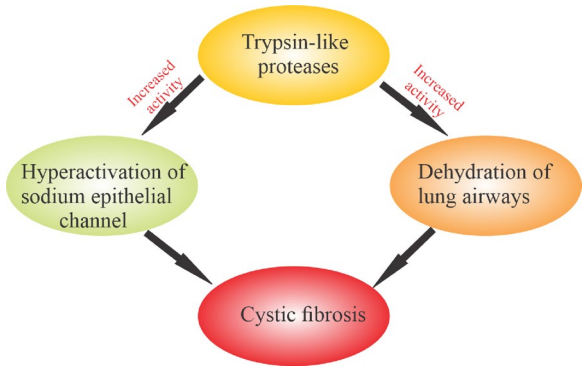

Fig. 2 The Role of Trypsin-like Proteases in the onset of Cystic fibrosis Trypsin-like proteases play a role in lung diseases by activating epithelial sodium channel which in turn causes dehydration in the lung airways [22]

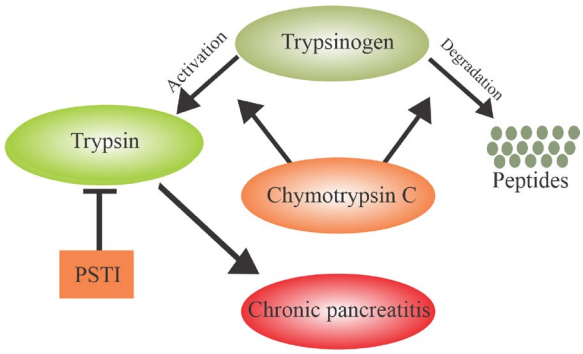

Fig. 3 Involvement of Trypsin in Chronic Pancreatitis. Trypsinogen can be covalently activated to active trypsin. The activity of the trypsin can be modulated by PSTI. Uncontrolled activity of trypsin can cause pancreatitis [25]. PSTI, Pancreatic Secretory Trypsin Inhibitor

the activity of trypsin is properly controlled by the pancreatic secretory trypsin inhibitor (PSTI). The PSTI is also known as the serine protease inhibitor kazal type 1 (SPINK 1), a protein that inhibits trypsin and is produced in the acinar cells of the pancreas [23]. PSTI inactivates the amount of trypsinogen that is being converted to trypsin thereby preventing the damage of the acinar cells of the pancreas. In some certain conditions, the activation of trypsin (ectopic activation) exceeds the capacity of the PSTI which subsequently leads to the activations of other downstream zymogens and then ultimately the digestion of the pancreas [24].

An observation that the mutation of Serine Protease 1 (PRSS1) gene also known as the cationic trypsinogen (CT) gene, that encodes the cationic trypsinogen, causes hereditary pancreatitis, shows the role of trypsin in the development of pancreatitis [25, 26]. The mutation in PRSS1 gene leads to increase in the activity of trypsin [24]. Due to inadequate ductal flushing, mutations in the cystic fibrosis transmembrane conductance regulator (CFTR), a chloride bicarbonate channel found on the ductal epithelium, can also increase pancreatitis risk [25].

In addition to trypsin inhibition, the degradation of trypsinogen by the combined activities of chymotrypsin C (CTRC) and trypsin provides an independent line of defense against trypsinogen autoactivation in the pancreas. The presence of active trypsin is required for the degradation mediated by CTRC, first to activate chymotrypsinogen C to active CTRC, and secondly to enhance degradation by autolytic breakage of the Arg122-Val123 peptide link in trypsinogen. As a result, after the SPINK1-dependent defense mechanism has been compromised, protective trypsinogen breakdown should proceed [25]. Trypsin inhibition or trypsinogen degradation is two protective mechanisms in the pancreas that prevent trypsinogen activation and thereby lower trypsin activity (Fig. 3).

There are currently no viable preventative or treatment options for this condition, owing to a lack of an optimal animal model that closely resembles the human version of the disease and a lack of knowledge of its pathophysiology [27].

Viral diseases Viral diseases are caused by viral infections. Viruses are able to change rapidly and adapt to new environmental conditions. Viral infections are spread through contact with infected fluids or materials and vaccination is a known method to control viral spread. Proteases are known to enhance viral infections [28]. Some of the well-known viruses that have caused serious epidemics with involvement of trypsin in the pathogenesis of the diseases include:

(i) Severe acute respiratory syndrome (SARS-CoV-2)

A novel coronavirus was identified in 2019, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This virus is the causative agent of coronavirus disease, COVID-19, a disease that affects the respiratory system. It became a global pandemic and a threat to healthy living because of its fast spread. SARS-CoV-2, closely related to SARS-CoV, is the seventh human coronavirus identified. It belongs to the family Coronaviridae, genus Betacoronavirus and subgenus Sarbecovirus. SARS-CoV-2 is an RNA virus that possesses RNA genome encoding 16 non-structural proteins (nsp1- nsp16) and 4 canonical structural proteins: envelope, spike, nucleocapsid and membrane proteins [29, 30].

Generally, coronaviruses enter cells by cytoplasmic or endosomal membrane fusion. Trypsin has been found to have a positive effect on the fusion of both animal

and human coronaviruses [31, 32]. Using Vero E6 cells as a case study, the effect of trypsin on viral replication was studied and for the replication of SARS-CoV-2, it was measured through the monitoring of intensity of cytopathic effect (CPE) and it was noted that exogenous trypsin had no effect on SARS-CoV-2 during a one-hour inoculation period. CPE observations showed that when trypsin is added to the medium after infection, SARS-CoV-2 infectivity increases. When trypsin was added at different time points after infection to Vero E6 cells, a considerable enhancement of infectivity only at early stages of viral infection was discovered which suggests that trypsin plays a role in entry pathway of SARS-CoV-2. The action of trypsin on SARS-CoV-2 viral internalization was also studied using Vero E6 cells as case study and after critical examination of the two phases of viral entry- attachment and penetration, findings suggest that treatment of cells with trypsin enhances SARS-CoV-2 internalization [32].

Several proteases, including trypsin facilitate entry of SARS-CoV-2 by attachment of viral particles to surface of the cell through cell receptors and viral spike protein interaction at the cell membrane either via the endosomal pathway or non-endosomal pathway. The route taken is dependent on the presence of proteases like trypsin. When trypsin treatment is done in a post-attachment step (internalization), the SARS-CoV-2 replication is enhanced and it leads to severe damage of target organs. These findings show trypsin treatment facilitated in vitro viral growth [32].

(ii) Influenza A virus

Influenza A virus (IAV) is the most prevalent pathogen in human which causes considerable morbidity and mortality every year in babies and the elderly. The highly pathogenic avian IAV (HPA1) H5, H7 and H9 subtypes cause systemic multiple organ failure (MOF) with significant fatality rate in humans [33, 34]. Hemagglutinin precursor (HA0), the viral envelope from glycoprotein, is converted proteolytically into HA1 and HA2 subunits by the host cellular trypsin type serine proteases in the process of IAV infection and proliferation. The process is a prerequisite for viral membrane fusion activity [34].

Through proteinase-activated receptor-2 (PAR-2) pathways, the trypsin potentiates cause the cellular dysfunction and fluid imbalance which finally results in multiple organ failure (Fig. 4). "Influenza virus-cytokine trypsin" cycle is a term that describes the close intermediates among IAV infection, cytokines and cellular ectopic trypsin to induce influenza pathogenicity. Interleukin-1β (1L-1β) also has a role to play in the pathogenicity of influenza-related multiple organ failure; it functions as a key component of the cytokine storm that induces the

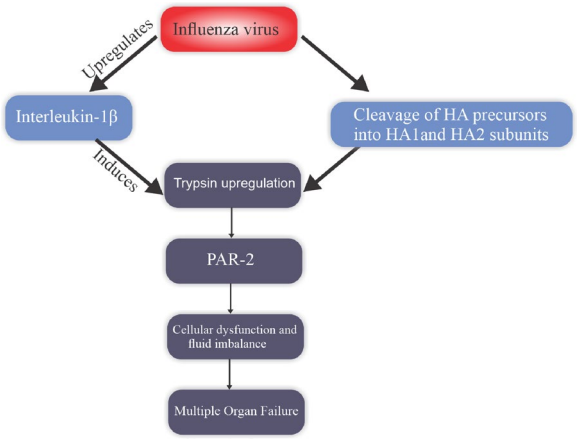

Fig. 4 Trypsin Upregulation in Influenza virus-induced Multiple Organ Failure. Influenza virus upregulates the cytokine Interleukin-1β which induces the upregulation of trypsin. Trypsin helps in the viral replication by cleaving HA precursor into HA1 and HA2 subunits. In addition, trypsin through PAR-2 pathways causes the cellular dysfunction and fluid imbalance which finally leads to multiple organ failure [34]. HA, hemagglutinin; PAR-2, proteinase activated receptor-2

upregulation of ectopic trypsin in the cycle. The cycle is also linked to 'metabolic disorders-cytokine' [34]. Bovine and porcine trypsin help in the virus replication of influenza A (also known as H1N1 virus) in S1-6 cell [35]. Trypsin plays a crucial role in the production of influenza virus in cultured cells by facilitating the cleavage of hemagglutinin (HA0) into its two disulfide-bonded subunits, HA1 and HA2, which is essential for virus replication [35, 36]. Additionally, trypsin inhibits the expression of the myxovirus resistance 1 (Mx1) gene, which is stimulated by interferon and contributes to more efficient influenza virus replication [35, 37].

Porcine deltacoronavirus (PDCoV) PDCoV belongs to the genus deltacoronavirus and family of coronaviridae. It is a newly rising swine pathogen. It was first reported in Hong Kong in 2012 and it is now epidemic in several countries [38]. It has also been shown to infect chicken and calves [39]. In recent studies, Porcine aminopeptidase N (pAPN) has been found to be a functional receptor of PDCoV [40, 41]. Trypsin was first used when PDCoV was first isolated in the swine testicular (ST) and LLC Porcine Kidney (LLC-PK cells) [42]. Its role in the lifecycle of the virus has not been found though it was used in the isolation and propagation of PDCoV [31].

Trypsin does not promote the entry of the PDCoV and it does not influence the virus release. However, it is thought to play a role in cell-to-cell membrane fusion mediated by PDCoV thereby facilitating the spread of the virus [31]. The addition of exogenous trypsin to cell cultures is necessary in the isolation and propagation of

PDCoV and porcine epidemic diarrhea virus (PEDV) and it is generally accepted that trypsin is essential and not indispensable for all strains of PEDV in Vero cells [31].

Inflammation Trypsin also plays important role in some health conditions including inflammation, wound healing and burn injuries. In some cases, it has been deployed as chemotherapeutic agent.

(i) Wound healing

There are four stages in the healing of wounds: hemostasis and coagulation, inflammation, proliferation and remodeling [43]. Generally, trypsin can play an anti-inflammatory role to treat or reduce inflammation but it is used most commonly with chymotrypsin. Uncontrolled inhibition of several protease inhibitors like alpha1-antitrypsin (A1AT) that follows an acute injury can lead to inflammation [43]. This early stage of tissue damage causing an inflammatory response can be treated with trypsin: chymotrypsin.

Postulated mechanisms have shown that after early stages of inflammation is controlled by trypsin, chymotrypsin and trypsin make available plasmin for fibrinolysis and blood clotting, it then helps to maintain A1AT levels which in turn reduces degradation effected by proteolytic enzymes and this further establishes the anti-inflammatory effect. This helps to reduce the pain associated with healing and also speeds up the healing process [43].

(ii) Burn injuries

Although the healing of burn injuries generally follows the same pattern of tissue repair—hemostasis and coagulation, inflammation, proliferation and scar formation/remodeling, burn injuries are still part of the most severe injuries body tissues can suffer because they are associated with generalized edema and also, hypovolemic shock—a condition where the heart cannot pump enough blood [43, 44]. The repair process after burn injuries can be hastened when tissue destruction is reduced—inflammation and edema being reduced and the trypsin: chymotrypsin helps in this process by minimizing tissue destruction induced by proteases. Higher levels of proteolytic inhibitors are maintained (α_1 antitrypsin and α_2 macroglobulin) which in turn help to minimize tissue degradation [43].

The effects of the trypsin: chymotrypsin treatment is seen mainly in acute-phase proteins (C-reactive protein, alpha-1 antitrypsin and alpha-2 macroglobulin). This treatment shows an increase in acute-phase proteins at the initial stage of treatment, then a slight decline in the C-reactive protein which shows the anti-inflammatory efficacy of this combination. Healing of burn injuries is facilitated by the decrease in the rate of inflammation, edema and tissue damage. This decrease arises from the maintenance of high levels of protease inhibitors through reduced protease-induced tissue degradation by the enzyme complex. This reduction in tissue degradation leads to a corresponding decrease in formation of free radicals and an increase in duration for the maintenance of higher antioxidant levels [43].

(iii) Sciatica (lumbar radiculopathy)

Sciatica is a painful condition associated with inflammation occurring from the lower back downwards to the legs. Sciatica is caused by hernia or protrusion of the invertebral disc which results in inflammation and compression of the root of the spinal nerve. Because of the anti-inflammatory effect of trypsin, it has been used in combination with chymotrypsin to improve and reduce symptoms [43, 45]. Gaspardy et al. [45] carried out a survey on how trypsin helps in treatment of sciatica and results suggested that trypsin, when used in combination with chymotrypsin, helps to treat symptoms of sciatica that results from hernia of intervertebral disc by reducing inflammatory edema (dropsy) in the nerve roots. The mechanism of action of trypsin in this case is however not fully understood.

Chymotrypsin

Structure, localization and mechanism

Chymotrypsin (EC 3.4.21.1) is a pancreatic serine protease, a digestive enzyme that functions in the duodenum, where it breaks down protein and polypeptides (proteolysis). Chymotrypsin cleaves at the N-terminal of aromatic amino acid residues (tyrosine, tryptophan and phenylalanine) of peptides.

Role of chymotrypsin in diseases and as chemotherapeutic agents

a. Inflammation

i. Wound healing and tissue repair

Chymotrypsin is widely used to help speed up the repair of traumatic, surgical, and orthopedic injuries. Its anti-inflammatory, antioxidant, and anti-infective properties help resolve inflammation caused by injury and help facilitate the healing process. Chymotrypsin can either be used singly or in combination with other enzymes with proteolytic action like trypsin (Fig. 5) [43].

α-Chymotrypsin is a pancreatic enzyme naturally obtained from bovine pancreas, and it has the proteolytic characteristic of reducing inflammation. It works by inhibiting chronic fibrin formation and this in turn

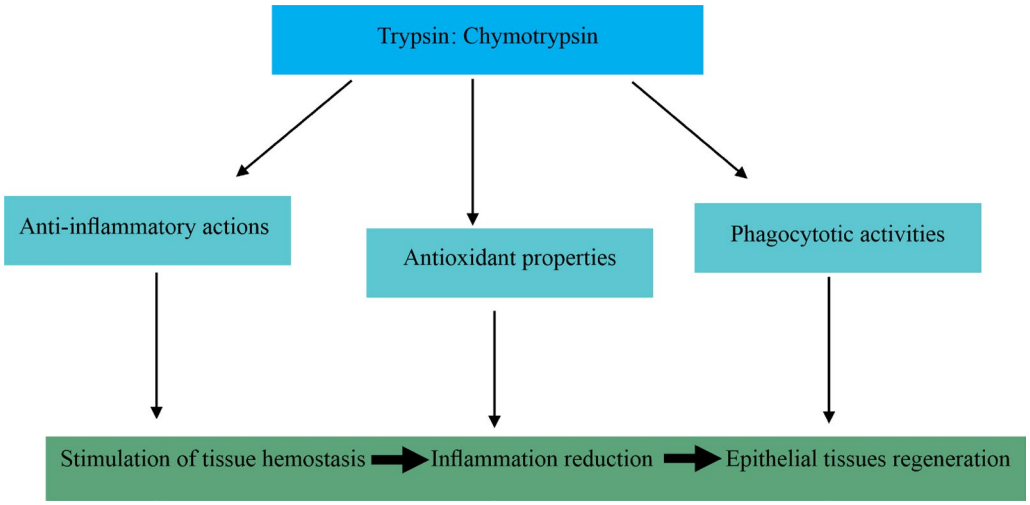

Fig. 5 Trypsin: Chymotrypsin in Body Physiology. Trypsin/chymotrypsin combination has been deployed as anti-inflammatory chemotherapeutic agent in wound healing [43]

helps to prevent the reabsorption of hematoma and the formation of edema. This prevents local circulation disorders and helps re-establish continuity of normal structures. Chymotrypsin accelerates wound healing and good scar tissue formation by stimulating tissue homeostasis, a process through which epithelial tissues are regenerated. It also helps in wound healing by cleaning areas of necrotic debris, a process required to maintain homeostasis in multicellular organisms [46].

ii. Arthritis

Arthritis is an inflammation of one or more joints which leads to pain and stiffness. There are more than hundred different forms of arthritis. Rheumatoid, osteoarthritis and psoriatic arthritis are the most common [47]. Rheumatoid arthritis is a complex inflammatory disorder, and it is typically associated with joint swelling, pain, synovial inflammation and stiffness [48]. Chymotrypsin plays a significant role in the metabolism of organism and affects cell proliferation and apoptosis. Abnormality in the chymotrypsin levels causes different diseases such as inflammatory arthritis, diabetes, pharyngitis and pancreatic cancer [49]. Chymotrypsin and cathepsin catalyze the cleavage of Interleukin 1-β (IL -1β) precursor into active IL -1β leading to arthritis. Excessive production of IL -1β also drives inflammation in mouse models [48].

b. Antibacterial activity

Antibiotics are used to treat bacterial infection and the rise of drug-resistant bacteria has limited their usage. Although, novel antibiotics are being discovered but resistance emerges after a short period. To solve this problem, there is a need to develop new antimicrobial agents [50, 51]. Some serine proteases have been found to be part of new antimicrobial agents. For instance, an ovary-specific trypsin-like protease, Ovochymase in amphioxus *Branchiostoma belcheri* with antibacterial activity has been reported [52].

Neutrophil serine proteases (NSPs) are important for the effective functioning of neutrophil which can rapidly kill bacteria using the mechanisms that depend on the antimicrobial granular component [53]. The activity of chymotrypsin in bacteria growth is mainly dependent on the concentration of chymotrypsin and the strain of bacteria. In a study by Zhou et al. [50], both gram-positive and gram-negative bacteria examined reacted to chymotrypsin in different ways, which might be related to their structural differences.

Gram-negative bacteria have an outer membrane which is chemically distinct from the plasma membrane. The presence of this membrane may be responsible for their protection against chymotrypsin destruction while the absence of an outer membrane renders gram-positive bacteria susceptible to the destructive forces of chymotrypsin [50]. Low concentrations of chymotrypsin can help gram-negative bacteria grow faster by activating protein-activated receptors (PARs). Chymotrypsin also digests extracellular protein and facilitates the nutrients which are absorbed by bacteria. Chymotrypsin has been shown to have elastase activity. The antimicrobial elastase activity of chymotrypsin in the dermis can be used to maintain skin elasticity which can be used as a cosmetic ingredient to prevent skin aging [54].

c. Chymotrypsin-like Proteases in SARS-CoV-2

Chymotrypsin-like proteases play a crucial role in the life cycle of many viruses, including coronaviruses, where they are involved in the processes of viral replication and maturation. Among these, the chymotrypsin-like protease ($3CL^{Pro}$), also known as the main protease (M^{Pro}), is essential for the cleavage of the viral polyprotein into functional nonstructural proteins that are critical for the replication of SARS-CoV-2 and other coronaviruses. Due to its indispensable function in viral replication, $3CL^{Pro}$ has been recognized as a promising therapeutic target in the development of antiviral drugs aimed at controlling SARS-CoV-2 infections.

Structurally, the $3CL^{Pro}$ of SARS-CoV-2 is composed of three domains. Domain I and domain II share structural similarities with the serine protease chymotrypsin, featuring a conserved fold that facilitates the proteolytic activity. These domains form a catalytic dyad composed of cysteine and histidine residues, which are vital for the enzyme's function. Domain III, in contrast, consists of a cluster of alpha-helices and is believed to play a role in dimerization, which is necessary for the protease's enzymatic activity. The unique structure of $3CL^{Pro}$ distinguishes it from human proteases, making it an attractive target for selective inhibition without affecting host proteases [55].

Inhibition of $3CL^{Pro}$ activity has emerged as a strategy to halt viral replication and suppress the spread of infection. Several antiviral compounds, including those already approved by the Food and Drug Administration (FDA), such as remdesivir and ritonavir, have been investigated for their efficacy against SARS-CoV-2. These nucleotide analogs primarily act by inhibiting the viral RNA polymerase, but studies have shown that they can also interfere with $3CL^{Pro}$ activity, reducing viral replication [30].

Moreover, computational approaches, such as molecular docking and high-throughput virtual screening, have been instrumental in identifying potential inhibitors of $3CL^{Pro}$. These studies have revealed several promising candidates, including both repurposed drugs and novel compounds, that exhibit strong binding affinity to the active site of $3CL^{Pro.}$ The development of specific inhibitors targeting $3CL^{Pro}$ could offer an alternative therapeutic approach to managing SARS-CoV-2 and other coronavirus infections. Ongoing research continues to explore small-molecule inhibitors and peptide-based therapies that could effectively block $3CL^{Pro}$ function and provide a new line of defense against COVID-19 [56].

Despite significant progress in identifying potential inhibitors, challenges remain in optimizing these compounds for clinical use. Issues such as drug stability, bioavailability, and potential side effects must be carefully evaluated to ensure the safety and efficacy of these therapies. Nevertheless, the inhibition of $3CL^{Pro}$ represents a promising avenue for therapeutic intervention, offering hope for more effective treatments against SARS-CoV-2 and future coronavirus outbreaks.

d. Regulation of calcium ion in the blood

The thyroid gland and parathyroid hormone (PTH) maintain calcium homeostasis. Calcium homeostasis in the blood is important for physiological function. Chymotrypsin C was initially recognized as a serum calcium-decreasing factor in the pancreas. This role of chymotrypsin C as a serum calcium decreasing factor requires the trypsin-mediated activation of the protein by cleaving the pro-peptide converting chymotrypsin C to its active form [57]. Chymotrypsin C is known with the ability to suppress receptor-Kb ligand (RANKL) and induce calcium oscillation pathways in osteoclasts [58].

In 2012, Tomomura et al. [57] investigated the effect of chymotrypsin C on the function of mature osteoclasts by treating with receptor activator of NF-Kb ligand (RANKL). Chymotrypsin C inhibited RANKL-stimulated bone resorptive activity of mature osteoclasts. In bone marrow cell cultures, chymotrypsin C had no effect on macrophage colony formation from monocyte lineage cells or osteoclast progenitor regeneration. Chymotrypsin C inhibited accumulation of the RANKL-stimulated Nuclear Factor of Activated T-cells (NFATc) in bone marrow cells, which is a key transcription factor for the differentiation of osteoclasts. During osteoclasts differentiation (osteoclastogenesis), the receptor activator of NFkB ligand (RANKL) induces Ca^{2+} oscillations and activates the Nuclear Factor of activated T-cells 1 (NFATc1) [59].

e. Chymotrypsin in pancreatic *cancer* and its anti-tumor effect

Pancreatic cancer is a very deadly disease with a fast rate of metastasis and the lack of early detection makes it deadlier. Radiotherapy and chemotherapy are the known methods for pancreatic cancer management, but because of the undesirable side effects, new less invasive treatments are being sought for [60]. Studies have shown that chymotrypsin has an effect on the activity of the cells that are linked to the immune system [61]. The anti-inflammatory effects of pancreatic enzymes, and also the reduction of phagocytic and microbial activity on the immune system through the interaction of proteases with α 1-antitrypsin and α 2-macroglobulin, may reinforce their anti-tumor effect. The anti-tumor effect of chymotrypsinogen

just like other pancreatic proenzymes are clear, but the mechanism of action is still under investigation [62].

The anti-tumor therapy of chymotrypsin involves the participation of all the isoforms of chymotrypsin [62]. Chymotrypsin can be extracted from porcine or bovine pancreatic juice and has been successfully tested to play an active role in anti-inflammation and immune system strengthening, and these are critical events in tumorigenesis. Several published works have shown the efficacy of pancreatic enzymes in the treatment of tumors and cancers and how safe they compared to other treatment options. Treatment with pancreatic enzymes also decreased the possible recurrence of the disease in the long term and increased the survival rate of patients on the average [62]. Chymotrypsin, just like other pancreatic enzymes decreases the frequency of metastasis of malignant tumors such as breast cancer, multiple myeloma, head and neck cancer, colorectal cancer, pancreatic adenocarcinoma and pancreatic cancer [63]. In 2001, Dale et al. [64] carried out procedures that showed that pancreatic enzyme therapy like chymotrypsin helped to reduce the symptoms of radiotherapy when used as an adjuvant although there were a few limitations to the procedures. The study did not analyze the effect of the sole treatment on the patient's survival rate. Studies have shown that enzyme therapy when used together with other chemotherapy or radiotherapy, reduces the side effects that might come with the treatment option and thus have a general positive impact on the patients [62].

Chymotrypsin was previously shown to play a role in apoptosis of cancer cells but its effect on cell migration ability was unclear. In a study involving 26 identified differential proteins, cytokeratin 18 was found to be the most closely associated with chymotrypsin C. Cytokeratin 18 is present in carcinomas and also expressed in tissues in the developmental stages of cancer. Overexpression of chymotrypsin C in Aspc-1 pancreatic cells was associated with downregulation of cancer cell migration ability and vice versa. Wang et al. [65] showed the effects of chymotrypsin C on cancer cells motility. Upregulation of cytokeratin 18 was shown to be involved in cancer metastasis [66]. Transwell test and cell migration assay show that overexpression of chymotrypsin C results in suppression of pancreatic cancer cell migration [65].

Elastase

Elastases (EC 3.4.21.36) are a family of serine proteases that degrade elastin and have a wide range of substrate specificity. Elastase was discovered in 1950 by Balo and Banga and is known for its capacity to hydrolyze the aorta's elastin filaments. Elastase is found in the pancreas and pancreatic juice of numerous mammals and birds, as well as human serum, granulocytes, and erythrocytes, in *Flavobacterium elastolyticum, Clostridium histolyticum,* and *Staphylococcus epidermis* [67]. Overexpression of elastase, just like other serine proteases could lead to several health conditions, just as under expression could also disrupt overall healthy living. To avoid overexpression, elastase is irreversibly inhibited by alpha-1- antitrypsin (A1AT), an acute protein which binds to the active site of not only trypsin, but also elastase. Uncontrolled elastase activity contributes to human pathology by disrupting tight junctions, causing tissue damage by its proteolytic actions, breaking down of cytokines and cleaving of immunoglobulin A (IgA), immunoglobulin B (IgB), complement receptor type 3 (C3bi), and complement receptor (CR1). This cleavage participates in the decrease in neutrophilic ability to kill bacteria by the process of phagocytosis [68, 69].

Role of elastase in disease conditions

a. Emphysema

Emphysema causes the air sacs in the lungs (alveoli) to be damaged. Over time, the inner walls of the air sacs weaken and rupture, resulting in larger air gaps rather than a large number of small ones. Both neutrophil and macrophage elastases has been linked to the onset and progression of lung illness. The elastin-rich connective tissue framework of the lungs appears to be particularly susceptible to the action of elastolytic proteases, despite the fact that it is not likely that the reason these proteases evolved is to damage lung tissue. Assuming that neutrophil elastase is involved in neutrophil migration near a site of inflammation and the destruction of proteins from attacking organisms or other inflammatory response products, inhibitors of this protease protect normal tissues from its catalytic action. Alpha-1 antitrypsin deficiency results in disturbance of protease-anti-protease equilibrium and increases the risk of destructive lung disease (Fig. 6) [70].

Emphysema can manifest itself in a variety of ways in people, including centrilobular, panacinar, paraseptal, and airspace expansion with fibrosis. These types of emphysema have different morphologic and background properties, implying that their pathophysiology is different. Human neutrophil elastase and other elastolytic enzymes can cause experimental emphysema, while nonelastolytic proteases cannot. In the blotchy mouse, disruption of elastic fibers appears to be the underlying characteristic of lathyrogen-induced airspace expansion and emphysema. However, in cadmium-induced airspace enlargement with fibrosis or emphysema linked with hyperoxia or severe malnutrition, there is no evidence of elastic fiber damage. As a result, not all kinds

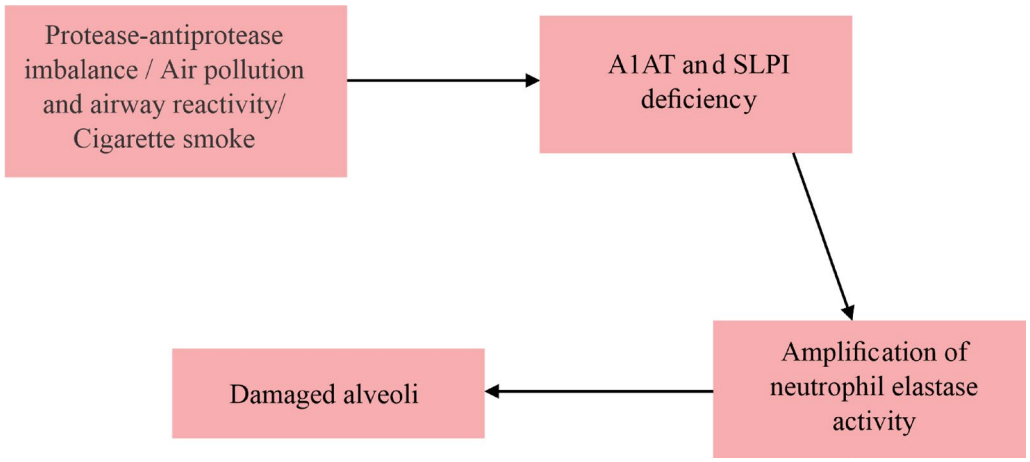

Fig. 6 Pathway of Tissue Damage Leading to Emphysema. Cigarette smoke and pollutants in the air inhibits α-1 antitrypsin and other protease inhibitors resulting in high neutrophil elastase activity thus resulting in lung damage [73]. A1AT: Alpha 1 anti-trypsin; SLPI: Secretory leukocyte protease inhibitor)

of experimental emphysema have elastic fiber disruption [71].

The difference between emphysema and cystic fibrosis is not as clear as previous studies suggested. These two diseases can coexist in human lungs and mice who have been exposed to cigarette smoke or that have been infused with elastolytic enzymes or bleomycin. Studies show that emphysema is caused by a protease or antiprotease imbalance, and antiproteases may play a role in modulating the fibrotic process. It has been shown in experimental animals that neutrophil elastase is a common pathogenic connection between cystic fibrosis and emphysema [72].

Elastin-degrading enzymes play a crucial role in the disorganization of elastic fiber networks within the lungs, which can lead to emphysema in experimental animal models. In the lungs, neutrophils and alveolar macrophages serve as key sources of endogenous elastases. Neutrophil elastase, an intracellular enzyme associated with granules, is typically regulated by alpha-1 antitrypsin, a protease inhibitor. However, when this regulation is impaired, such as in cases where the enzyme is not sufficiently inhibited, it can result in the development of emphysema, as observed in experimental mice. A newer discovery, macrophage elastase, is less effectively inhibited by alpha-1 antitrypsin and is not associated with granules. Research has shown that macrophages from cigarette smokers produce elastase in culture, and cigarette smoke can inhibit the activity of elastase inhibitors, suggesting that elastases contribute to the pathogenesis of emphysema in humans [73]. The current treatment strategies for emphysema focus on managing symptoms rather than curing the disease. These include bronchodilator inhalers to open the airways, corticosteroids to reduce inflammation, and oxygen therapy. Additionally, individuals with emphysema are more susceptible to respiratory infections, making the use of antibiotics a common requirement to prevent complications.

Interestingly, natural products have gained attention for their potential therapeutic effects on emphysema. One such example is *Rubus rosifolius*, commonly known as rose leaf bramble. This prickly perennial plant, with a woody stem, is native to rainforests and tall open forests in the Himalayas, eastern Australia, and East Asia. It is also found in the wild in the highlands of Puerto Rico and Indonesia. Studies have shown that *Rubus rosifolius* possesses elastase-suppressing properties. Methanol extracts of this plant have been identified as potential elastase inhibitors, with ellagic acid being the active compound responsible for this inhibitory activity [74]. These findings suggest that *Rubus rosifolius* could be explored further for its potential in the development of novel treatments for emphysema.

b. Chronic obstructive pulmonary disease (COPD)

Chronic obstructive pulmonary disease (COPD) is a global health problem that affects about 300 million people globally and kills roughly three million people every year. It develops as a result of cigarette smoking or inhalation of environmental and occupational contaminants, such as high quantities of dust, as in mineshaft, and certain gases, or as a result of genetic abnormalities for example alpha 1-antitrypsin deficiency. Increased numbers of macrophages, neutrophils, B- and T-lymphocytes in the airways and lung parenchyma characterize chronic

inflammation in COPD patients, and there is growing evidence that these cells participate in directing the inflammatory response in COPD [75].

The rate of lung function decline and peripheral airway dysfunction have been demonstrated to be related to sputum neutrophil levels. Acute bronchitis caused by viral or bacterial infections is the leading cause of morbidity and death in COPD patients with airway disease, and it is linked to high NE levels. The protease–anti-protease activity in the COPD airway is controlled by NE and other proteases working together. Macrophage elastase (MMP-12), for example, is essential for emphysema following smoke exposure, ([76, 77]. NE causes emphysema by activating matrix metalloproteinase and cysteinyl cathepsins, and it keeps MMP activity going by degrading tissue inhibitor of matrix metalloproteinase-1 (TIMP-1), a key inhibitor of MMPs. The oxidation and inactivation of alpha-1-antitrypsin by reactive oxygen species leads to unrestricted NE activity. The release of neutrophil extracellular traps into the airway milieu is triggered by the presence of NE and myeloperoxidase in the body. The presence of neutrophil extracellular traps (NETs) in the chronic obstructive pulmonary disease (COPD) airway is linked to lower lung function, higher exacerbations, and impaired neutrophil phagocytosis. The consequences of NE are most noticeable in alpha-1-antitrypsin deficiency, where lower levels or complete lack of alpha-1-antitrypsin lead to uninhibited NE activity and consequent alveolar matrix degradation. There is a synergy between NE and MMP-12 (macrophage elastase) which promote tobacco smoke-induced COPD lung pathology and also results in unrestrained protease activities [77].

c. Cystic fibrosis

Cystic fibrosis is a respiratory disease that affects the lungs and also the pancreas, kidneys, liver and intestine [78]. This respiratory disease is mostly attributed to genetic disorders and is characterized by frequent lung infections and abdominal mucus, cysts within the pancreas, hypoxia and inflammation of the airways [77]. Sputum from cystic fibrosis patients contains elevated level of neutrophil serine proteases. In a healthy individual, it aids in the phagocytic process by destroying microbial peptides and they are kept in equilibrium by cognate anti-proteases [79].

Chronic lung disease causes morbidity and death which is due to infection and inflammation of the airway that is a characteristic of cystic fibrosis as it has been shown that neutrophil elastase is present in the airway very early in the life of cystic fibrosis patient and appears as a biomarker of disease progression, predicting lung function decline and a condition in which the lung's airways become damaged, making it hard to clear mucus (bronchiectasis). The pro-inflammatory state of senescence in bronchial epithelial cell in vitro and potentially in vivo has been shown to be caused by elastase [80].

Neutrophil elastase is involved in the etiology of mucus hypersecretion, airway inflammation and reduced defenses against *Pseudomonas aeruginosa* infection, aside from structural damage to airwall. Furthermore, increased neutrophil elastase concentration was found to block the cystic fibrosis transmembrane conductance regulator chloride channel and activate epithelial sodium channels exacerbating fundamental anion secretion and sodium ion absorption abnormalities in cystic fibrosis airways [81].

d. Elastase in wound healing and muscle elasticity

In studies using a human microvascular endothelial cell model, it was found that polymorphonuclear leukocytes (PMN) stimulated with lipopolysaccharide bound neutrophils (LPS) in combination with formyl-methionyl leucyl phenyl alanine (fMLP) or C5a caused significant endothelial cell lysis that was unaffected by scavengers of oxygen free radical. Exposure to purified human leukocyte elastase (HLE) alone caused cell disintegration with a comparable time course of harm that was unaffected by scavengers of reactive oxygen species. A serine protease inhibitor prevented injury caused by human leukocyte elastase alone or by activated neutrophils, implying that HLE plays a significant aspect for leukocyte elastase in neutrophil mediated endothelial cell injury. Evidence suggests that unrestricted elastase activity is linked to wound healing delays. Increased elastase activity and tissue inflammation were seen in recombinant animals lacking the secretory leukoprotease inhibitor (SLPI) gene, as well as delayed wound closure in cutaneous wounds. In contrast to wild-type mice, SLPI-null mice wounds had higher levels of active transforming growth-factor beta than wild-type mice wounds. In SLPI-null animals, a neutralizing antibody to transforming growth factor-beta partly reduced inflammation and slowed wound healing [82].

Studies on the anti-elastase and anti-collagenase potential of Lactobacilli exopolysaccharide (LEPS) on normal human fibroblast shows that it could be a good choice of skin anti-aging agent for skin regeneration and tissue engineering due to their high anti-elastase, anti-collagenase, anti-oxidant and wound healing properties [83].

The tendon fascicles are held together by the interfascicular matrix (IFM). Its low stiffness behavior under modest stresses, allows non-uniform loading and increased overall flexibility of the tendon by permitting fascicle sliding. Because the interfascicular matrix (IFM)

is abundant in elastin, it was hypothesized that elastin depletion would have a major impact on IFM. For the first time, it was discovered that removing elastin from tendons affects the mechanical properties of the interfascicular matrix, lowering the recoverability and fatigue resistance, shedding light on the hierarchical mechanics of tendons, which is crucial for the development of innovative tendon damage treatment.

The contribution of particular proteins to tissue mechanical behavior can be used to identify key therapeutic targets. Tendon damage is growing more widespread and debilitating, yet there are currently no viable treatments. An examination into how elastin regulates tendon mechanical behavior was conducted using enzymatic digestion following elastase treatment and local mechanical characterization, and it was identified for the first time that the mechanical properties of the interfascicular matrix are affected by removing elastin from tendons, resulting in lower recoverability and fatigue resistance, which has shed light on the hierarchical mechanics of tendons, which is crucial for the development of innovative tendon damage treatments [84].

Conclusion

Extensive research has highlighted the critical roles of serine proteases, particularly trypsin, chymotrypsin, and elastase, in the onset and progression of various diseases. These enzymes not only contribute to the disruption of physiological homeostasis but, in some cases, offer therapeutic potential for treating certain conditions. Their importance as drug targets continues to grow due to their involvement in essential biological processes. The overexpression of these enzymes can lead to severe and potentially life-threatening diseases, which the body counteracts through the presence of protease inhibitors that regulate enzyme activity. Furthermore, their secretion as inactive zymogens, requiring proteolytic activation, serves as a crucial control mechanism to prevent unregulated protease activity. Although significant progress has been made in understanding these enzymes, many aspects of their roles in disease pathology remain unexplored, highlighting the need for continued research to fully realize their therapeutic potential.

Abbreviations

ECM	Extracellular matrix
MMPs	Matrix Metalloproteinases
PAR2	Protease-activated Receptor-2
GPCR	G-protein-coupled receptor
TLPs	Trypsin-like proteases
PSTI	Pancreatic secretory trypsin inhibitor
SPINK 1	Serine protease inhibitor kazal type 1
PRSS1	Serine Protease 1 gene
CFTR	Cystic fibrosis transmembrane conductance regulator
HA	Hemagglutinin

Acknowledgements

Not applicable.

Author contributions

Oladoyin Grace Famutimi contributed to methodology, investigation, data curation, visualization, formal analysis, writing-original draft preparation, writing-reviewing and editing. Victor Gbolahan Adebiyi and Bukola Grace Akinmolu helped in methodology, investigation, data curation, writing-original draft preparation. Omoniyi Vincent Dada was involved in methodology, investigation, data curation. Isaac Olusanjo Adewale contributed to conceptualization, validation, resources, supervision, funding acquisition, writing-reviewing and editing.

Funding

"This work was carried out under the COVID-19 Africa Rapid Grant Fund supported under the auspices of the Science Granting Councils Initiative in Sub-Saharan Africa (SGCI) and administered by South Africa's National Research Foundation (NRF) in collaboration with Canada's International Development Research Centre (IDRC), the Swedish International Development Cooperation Agency (Sida), South Africa's Department of Science and Innovation (DSI), the Fonds de Recherche du Quebec (FRQ), the United Kingdom's Department of International Development (DFID), United Kingdom Research and Innovation (UKRI) through the Newton Fund, and the SGCI participating councils across 15 countries in sub-Saharan Africa".

Availability of data and materials

The data used will be made available on request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All coauthors consented to the publication of the manuscript.

Competing interests

The authors declare no conflict of interest.

Received: 9 July 2024 Accepted: 12 September 2024
Published online: 17 September 2024

References

1. Garten W (2018) Characterization of proprotein convertases and their involvement in virus propagation. In: Böttcher-Friebertshäuser E, Garten W, Klenk HD (eds) Activation of viruses by host proteases. Springer International Publishing, Cham, pp 205–248
2. Seidah NG, Prat A (2012) The biology and therapeutic targeting of the proprotein convertases. Nat Rev Drug Discov 11(5):367–383. https://doi.org/10.1038/nrd3699
3. Long JZ, Cravatt BF (2011) The metabolic serine hydrolases and their functions in mammalian physiology and disease. Chem Rev 111(10):6022–6063. https://doi.org/10.1021/cr200075y
4. Schaller A, Stintzi A, Rivas S, Serrano I, Chichkova NV, Vartapetian AB, Martínez D, Guiamét JJ, Sueldo DJ, van der Hoorn RAL, Ramírez V, Vera P (2018) From structure to function—a family portrait of plant subtilases. New Phytol 218(3):901–915. https://doi.org/10.1111/nph.14582
5. Patel S (2017) A critical review on serine protease: Key immune manipulator and pathology mediator. Allergol Immunopathol 45(6):579–591. https://doi.org/10.1016/j.aller.2016.10.011
6. Barzkar N, Khan Z, Tamadoni Jahromi S, Pourmozaffar S, Gozari M, Nahavandi R (2021) A critical review on marine serine protease and its inhibitors: A new wave of drugs? Int J Biol Macromol 170:674–687. https://doi.org/10.1016/j.ijbiomac.2020.12.134

7. Kasperkiewicz P (2021) Peptidyl activity-based probes for imaging serine proteases. Front Chem 9:639410. https://doi.org/10.3389/fchem.2021.639410
8. Rakashanda S, Rana F, Rafiq S, Masood A, Amin S (2012) Role of proteases in cancer: A review. Biotechnol Mol Biol Rev 7(4):90–101. https://doi.org/10.5897/BMBR11.027
9. Kim KK, Turner R, Khazan N, Kodza A, Jones A, Singh RK, Moore RG (2020) Role of trypsin and protease-activated receptor-2 in ovarian cancer. PLoS ONE 15(5):e0232253. https://doi.org/10.1371/journal.pone.0232253
10. Lee K-H, Lee C-H, Jeong J, Jang A-H, Yoo C-G (2015) Neutrophil elastase differentially regulates interleukin 8 (IL-8) and vascular endothelial growth factor (VEGF) production by cigarette smoke extract. J Biol Chem 290(47):28438–28445. https://doi.org/10.1074/jbc.M115.663567
11. Yang Y, Wen Y, Cai YN, Vallée I, Boireau P, Liu MY, Cheng SP (2015) Serine proteases of parasitic helminths. Korean J Parasitol 53(1):1–11. https://doi.org/10.3347/kjp.2015.53.1.1
12. Lockett AD, Kimani S, Ddungu G, Wrenger S, Tuder RM, Janciauskiene SM, Petrache I (2013) α_1-Antitrypsin modulates lung endothelial cell inflammatory responses to TNF-α. Am J Respir Cell Mol Biol 49(1):143–150. https://doi.org/10.1165/rcmb.2012-0515OC
13. Elgendy A, Al-Ruweidi M (2016) A literature review on trypsin enzyme. Qatar University, College of Arts and Sciences, Department of Chemistry and Earth Science, pp. 4–5.
14. Soreide K, Janssen EA, Körner H, Baak JPA (2006) Trypsin in colorectal cancer: molecular biological mechanisms of proliferation, invasion, and metastasis. J Pathol 209(2):147–156. https://doi.org/10.1002/path.1999
15. Koistinen H, Kovanen R-M, Hollenberg MD, Dufour A, Radisky ES, Stenman U-H, Batra J, Clements J, Hooper JD, Diamandis E, Schilling O, Rannikko A, Mirtti T (2023) The roles of proteases in prostate cancer. IUBMB Life 75(6):493–513. https://doi.org/10.1002/iub.2700
16. Søreide K, Roalsø M, Aunan JR (2020) Is there a trojan horse to aggressive pancreatic cancer biology? A Review of the trypsin-PAR2 Axis to proliferation, early invasion, and metastasis. J Pancreat Cancer 6(1):12–20. https://doi.org/10.1089/pancan.2019.0014
17. Morán A, Iniesta P, García-Aranda C, De Juan C, Díaz-López A, Sánchez-Pernaute A, Torres AJ, Díaz-Rubio E, Balibrea JL, Benito M (2005) Clinical relevance of MMP-9, MMP-2, TIMP-1 and TIMP-2 in colorectal cancer. Oncol Rep 13(1):115–120
18. Jiang Y, Lim J, Wu K-C, Xu W, Suen JY, Fairlie DP (2021) PAR2 induces ovarian cancer cell motility by merging three signalling pathways to transactivate EGFR. Br J Pharmacol 178(4):913–932. https://doi.org/10.1111/bph.15332
19. Shi X, Gangadharan B, Brass LF, Ruf W, Mueller BM (2004) Protease-activated receptors (PAR1 and PAR2) contribute to tumor cell motility and metastasis. Mol Cancer Res 2(7):395–402
20. Scott A (2013) Cystic fibrosis. Radiol Technol 84(5):493–513 (**quiz 514–518**)
21. Kaur J, Singh PK (2022) Trypsin detection strategies: a review. Crit Rev Anal Chem 52(5):949–967. https://doi.org/10.1080/10408347.2020.1846490
22. Carroll EL, Bailo M, Reihill JA, Crilly A, Lockhart JC, Litherland GJ, Lundy FT, McGarvey LP, Hollywood MA, Martin SL (2021) Trypsin-like proteases and their role in muco-obstructive lung diseases. Int J Mol Sci 22(11):5817. https://doi.org/10.3390/ijms22115817
23. Sendler M, Lerch MM (2020) The complex role of trypsin in pancreatitis. Gastroenterology 158(4):822–826. https://doi.org/10.1053/j.gastro.2019.12.025
24. Hirota M, Ohmuraya M, Baba H (2006) The role of trypsin, trypsin inhibitor, and trypsin receptor in the onset and aggravation of pancreatitis. J Gastroenterol 41(9):832–836. https://doi.org/10.1007/s00535-006-1874-2
25. Hegyi E, Sahin-Tóth M (2017) Genetic risk in chronic pancreatitis: the trypsin-dependent pathway. Dig Dis Sci 62(7):1692–1701. https://doi.org/10.1007/s10620-017-4601-3
26. Demcsák A, Sahin-Tóth M (2024) Heterozygous spink1 deficiency promotes trypsin-dependent chronic pancreatitis in mice. Cell Mole Gastroenterol Hepatol. https://doi.org/10.1016/j.jcmgh.2024.05.009
27. Gui F, Zhang Y, Wan J, Zhan X, Yao Y, Li Y, Haddock AN, Shi J, Guo J, Chen J, Zhu X, Edenfield BH, Zhuang L, Hu C, Wang Y, Mukhopadhyay D, Radisky ES, Zhang L, Lugea A, Pandol SJ, Bi Y, Ji B (2020) Trypsin activity governs increased susceptibility to pancreatitis in mice expressing human PRSS1R122H. J Clin Invest 130(1):189–202. https://doi.org/10.1172/JCI130172
28. Lubinski B, Whittaker GR (2024) Host cell proteases involved in human respiratory viral infections and their inhibitors: a review. Viruses 16(6):984. https://doi.org/10.3390/v16060984
29. Shang J, Wan Y, Luo C, Ye G, Geng Q, Auerbach A, Li F (2020) Cell entry mechanisms of SARS-CoV-2. Proc Natl Acad Sci U S A 117(21):11727–11734. https://doi.org/10.1073/pnas.2003138117
30. Wang C, Horby PW, Hayden FG, Gao GF (2020) A novel coronavirus outbreak of global health concern. Lancet 395(10223):470–473. https://doi.org/10.1016/S0140-6736(20)30185-9
31. Yang Y-L, Meng F, Qin P, Herrler G, Huang Y-W, Tang Y-D (2020) Trypsin promotes porcine deltacoronavirus mediating cell-to-cell fusion in a cell type-dependent manner. Emerg Microbes Infect 9(1):457–468. https://doi.org/10.1080/22221751.2020.1730245
32. Kim Y, Jang G, Lee D, Kim N, Seon JW, Kim Y, Lee C (2022) Trypsin enhances SARS-CoV-2 infection by facilitating viral entry. Arch Virol 167(2):441–458. https://doi.org/10.1007/s00705-021-05343-0
33. Nakajima N, Van Tin N, Sato Y, Thach HN, Katano H, Diep PH, Kumasaka T, Thuy NT, Hasegawa H, San LT, Kawachi S, Liem NT, Suzuki K, Sata T (2013) Pathological study of archival lung tissues from five fatal cases of avian H5N1 influenza in Vietnam. Mod Pathol 26(3):357–369. https://doi.org/10.1038/modpathol.2012.193
34. Kido H, Takahashi E, Kimoto T (2019) Role of host trypsin-type serine proteases and influenza virus-cytokine-trypsin cycle in influenza viral pathogenesis. Pathogenesis-based therapeutic options. Biochimie 166:203–213. https://doi.org/10.1016/j.biochi.2019.09.006
35. Iskandar VI, Sasaki Y, Yoshino N, Abubakar RZR, Sato S, Muraki Y (2018) Optimization of trypsins for influenza A/H1N1 virus replication in MDCK SI-6 cells, a novel MDCK cell line. J Virol Methods 252:94–99. https://doi.org/10.1016/j.jviromet.2017.11.006
36. Garcia-Moro E, Zhang J, Calder LJ, Brown NR, Gamblin SJ, Skehel JJ, Rosenthal PB (2022) Reversible structural changes in the influenza hemagglutinin precursor at membrane fusion pH. Proc Natl Acad Sci U S A 119(33):e2208011119. https://doi.org/10.1073/pnas.2208011119
37. Seitz C, Isken B, Heynisch B, Rettkowski M, Frensing T, Reichl U (2012) Trypsin promotes efficient influenza vaccine production in MDCK cells by interfering with the antiviral host response. Appl Microbiol Biotechnol 93(2):601–611. https://doi.org/10.1007/s00253-011-3569-8
38. Woo PCY, Lau SKP, Lam CSF, Lau CCY, Tsang AKL, Lau JHN, Bai R, Teng JLL, Tsang CCC, Wang M, Zheng B-J, Chan K-H, Yuen K-Y (2012) Discovery of seven novel Mammalian and avian coronaviruses in the genus deltacoronavirus supports bat coronaviruses as the gene source of alphacoronavirus and betacoronavirus and avian coronaviruses as the gene source of gammacoronavirus and deltacoronavirus. J Virol 86(7):3995–4008. https://doi.org/10.1128/JVI.06540-11
39. Liang Q, Zhang H, Li B, Ding Q, Wang Y, Gao W, Guo D, Wei Z, Hu H (2019) Susceptibility of chickens to porcine deltacoronavirus infection. Viruses 11(6):E573. https://doi.org/10.3390/v11060573
40. Li W, Hulswit RJG, Kenney SP, Widjaja I, Jung K, Alhamo MA, van Dieren B, van Kuppeveld FJM, Saif LJ, Bosch B-J (2018) Broad receptor engagement of an emerging global coronavirus may potentiate its diverse cross-species transmissibility. Proc Natl Acad Sci U S A 115(22):E5135–E5143. https://doi.org/10.1073/pnas.1802879115
41. Wang B, Liu Y, Ji C-M, Yang Y-L, Liang Q-Z, Zhao P, Xu L-D, Lei X-M, Luo W-T, Qin P, Zhou J, Huang Y-W (2018) Porcine deltacoronavirus engages the transmissible gastroenteritis virus functional receptor porcine aminopeptidase n for infectious cellular entry. J Virol 92(12):e00318-e418. https://doi.org/10.1128/JVI.00318-18
42. Hu H, Jung K, Vlasova AN, Chepngeno J, Lu Z, Wang Q, Saif LJ (2015) Isolation and characterization of porcine deltacoronavirus from pigs with Diarrhea in the United States. J Clin Microbiol 53(5):1537–1548. https://doi.org/10.1128/JCM.00031-15
43. Shah D, Mital K (2018) The role of trypsin: chymotrypsin in tissue repair. Adv Ther 35(1):31–42. https://doi.org/10.1007/s12325-017-0648-y
44. Tiwari VK (2012) Burn wound: How it differs from other wounds? Indian J Plast Surg 45(2):364–373. https://doi.org/10.4103/0970-0358.101319
45. Gaspardy G, Balint G, Mitusova M, Lorincz G (1971) Treatment of sciatica due to intervertebral disc herniation with chymoral tablets. Rheumatol Phys Med 11(1):14–19. https://doi.org/10.1093/rheumatology/11.1.14

46. Ozdemir O, Ozkan K, Hatipoglu F, Uyaroglu A, Arican M (2016) Effect of asiaticoside, collagenase, and alpha-chymotrypsin on wound healing in rabbits. Wounds 28(8):279–286
47. Petchi RR, Vijaya C, Parasuraman S (2013) Anti-arthritic activity of ethanolic extract of Tridax procumbens (Linn.) in Sprague Dawley rats. Pharmacognosy Res 5(2):113–117. https://doi.org/10.4103/0974-8490.110541
48. Juma SN, Liao J, Huang Y, Vlashi R, Wang Q, Wu B, Wang D, Wu M, Chen G (2023) Osteoarthritis versus psoriasis arthritis: Physiopathology, cellular signaling, and therapeutic strategies. Genes Dis 11(3):100986. https://doi.org/10.1016/j.gendis.2023.04.021
49. Zou X, Zhao Y, Lai C, Liang Y, Lin W (2021) A non-peptide probe for detecting chymotrypsin activity based on protection-deprotection strategy in living systems. J Mater Chem B 9(40):8417–8423. https://doi.org/10.1039/d1tb01509e
50. Zhou D, Liu Z, Zhang D, Xu Y, Tan W, Ma L, Sun Y, Shen B, Zhu C (2013) Chymotrypsin both directly modulates bacterial growth and asserts ampicillin degradation-mediated protective effect on bacteria. Ann Microbiol 63(2):623–631. https://doi.org/10.1007/s13213-012-0512-x
51. Güell I, Micaló L, Cano L, Badosa E, Ferre R, Montesinos E, Bardají E, Feliu L, Planas M (2012) Peptidotriazoles with antimicrobial activity against bacterial and fungal plant pathogens. Peptides 33(1):9–17. https://doi.org/10.1016/j.peptides.2011.12.003
52. Gao K, Zhang S (2009) Ovochymase in amphioxus Branchiostoma belcheri is an ovary-specific trypsin-like serine protease with an antibacterial activity. Dev Comp Immunol 33(12):1219–1228. https://doi.org/10.1016/j.dci.2009.07.002
53. Stapels DAC, Ramyar KX, Bischoff M, von Köckritz-Blickwede M, Milder FJ, Ruyken M, Eisenbeis J, McWhorter WJ, Herrmann M, van Kessel KPM, Geisbrecht BV, Rooijakkers SHM (2014) Staphylococcus aureus secretes a unique class of neutrophil serine protease inhibitors. Proc Natl Acad Sci U S A 111(36):13187–13192. https://doi.org/10.1073/pnas.1407616111
54. Ambarwati NSS, Malik A, Listari AT, Nirwana N, Elya B, Hanafi M (2017) Antibacterial activity of fractions of ethyl acetate extract of *Garcinia lattissima* MIQ. fruits. Asian J Pharma Clin Res 10:81–84
55. Jiang X, Su H, Shang W, Zhou F, Zhang Y, Zhao W, Zhang Q, Xie H, Jiang L, Nie T, Yang F, Xiong M, Huang X, Li M, Chen P, Peng S, Xiao G, Jiang H, Tang R, Zhang L, Shen J, Xu Y (2023) Structure-based development and preclinical evaluation of the SARS-CoV-2 3C-like protease inhibitor simnotrelvir. Nat Commun 14(1):6463. https://doi.org/10.1038/s41467-023-42102-y
56. Khan SA, Zia K, Ashraf S, Uddin R, Ul-Haq Z (2021) Identification of chymotrypsin-like protease inhibitors of SARS-CoV-2 via integrated computational approach. J Biomol Struct Dyn 39(7):2607–2616. https://doi.org/10.1080/07391102.2020.1751298
57. Tomomura A, Bandow K, Tomomura M (2021) Purification and biological function of caldecrin. Medicines (Basel) 8(8):41. https://doi.org/10.3390/medicines8080041
58. Bandow K, Hasegawa H, Tomomura M, Tomomura A (2020) Caldecrin inhibits lipopolysaccharide-induced pro-inflammatory cytokines and M1 macrophage polarization through the immunoreceptor triggering receptor expressed in myeloid cells-2. Biochem Biophys Res Commun 523(4):1027–1033. https://doi.org/10.1016/j.bbrc.2020.01.045
59. Kajiya H, Okamoto F, Nemoto T, Kimachi K, Toh-Goto K, Nakayana S, Okabe K (2010) RANKL-induced TRPV2 expression regulates osteoclastogenesis via calcium oscillations. Cell Calcium 48(5):260–269. https://doi.org/10.1016/j.ceca.2010.09.010
60. Mun EJ, Babiker HM, Weinberg U, Kirson ED, Von Hoff DD (2018) Tumor-treating fields: a fourth modality in cancer treatment. Clin Cancer Res 24(2):266–275. https://doi.org/10.1158/1078-0432.CCR-17-1117
61. Biziulevicius GA (2006) Where do the immunostimulatory effects of oral proteolytic enzymes ('systemic enzyme therapy') come from? Microbial proteolysis as a possible starting point. Med Hypotheses 67(6):1386–1388. https://doi.org/10.1016/j.mehy.2006.05.051
62. González-Titos A, Hernández-Camarero P, Barungi S, Marchal JA, Kenyon J, Perán M (2021) Trypsinogen and chymotrypsinogen: potent anti-tumor agents. Expert Opin Biol Ther 21(12):1609–1621. https://doi.org/10.1080/14712598.2021.1922666
63. Chabot JA, Tsai W-Y, Fine RL, Chen C, Kumah CK, Antman KA, Grann VR (2010) Pancreatic proteolytic enzyme therapy compared with gemcitabine-based chemotherapy for the treatment of pancreatic cancer. J Clin Oncol 28(12):2058–2063. https://doi.org/10.1200/JCO.2009.22.8429
64. Dale PS, Tamhankar CP, George D, Daftary GV (2001) Co-medication with hydrolytic enzymes in radiation therapy of uterine cervix: evidence of the reduction of acute side effects. Cancer Chemother Pharmacol 47(Suppl):S29-34. https://doi.org/10.1007/s002800170006
65. Wang H, Sha W, Liu Z, Chi C-W (2011) Effect of chymotrypsin C and related proteins on pancreatic cancer cell migration. Acta Biochim Biophys Sin (Shanghai) 43(5):362–371. https://doi.org/10.1093/abbs/gmr022
66. Song J, Shih I, Chan DW, Zhang Z (2009) Suppression of annexin A11 in ovarian cancer: implications in chemoresistance. Neoplasia 11(6):605–614. https://doi.org/10.1593/neo.09286
67. Weiss FU, Budde C, Lerch MM (2016) Specificity of a polyclonal fecal elastase ELISA for CELA3. PLoS ONE 11(7):e0159363. https://doi.org/10.1371/journal.pone.0159363
68. Bieth JG (2001) The elastases. J Soc Biol 195(2):173–179
69. Abdulkarim A, Craig TJ (2024) Alpha-1 Antitrypsin Mutation. In: StatPearls. StatPearls Publishing, Treasure Island (FL)
70. Sandhaus RA, Turino G (2013) Neutrophil elastase-mediated lung disease. COPD 10(Suppl 1):60–63. https://doi.org/10.3109/15412555.2013.764403
71. Demkow U, van Overveld F (2010) Role of elastases in the pathogenesis of chronic obstructive pulmonary disease: Implications for treatment. Eur J Med Res 15(Suppl 2):27–35. https://doi.org/10.1186/2047-783X-15-S2-27
72. Lucattelli M, Bartalesi B, Cavarra E, Fineschi S, Lunghi B, Martorana PA, Lungarella G (2005) Is neutrophil elastase the missing link between emphysema and fibrosis? Evidence from two mouse models. Respir Res 6(1):83. https://doi.org/10.1186/1465-9921-6-83
73. Cha S-R, Jang J, Park S-M, Ryu SM, Cho S-J, Yang S-R (2023) Cigarette smoke-induced respiratory response: insights into cellular processes and biomarkers. Antioxidants 12(6):1210. https://doi.org/10.3390/antiox12061210
74. Desmiaty Y, Mulatsari E, Chany Saputri F, Hanafi M, Prastiwi R, Elya B (2020) Inhibition of pancreatic elastase in silico and in vitro by Rubus rosifolius leaves extract and its constituents. J Pharm Bioallied Sci 12(3):317–323. https://doi.org/10.4103/jpbs.JPBS_271_19
75. Spix B, Butz ES, Chen C-C, Rosato AS, Tang R, Jeridi A, Kudrina V, Plesch E, Wartenberg P, Arlt E, Briukhovetska D, Ansari M, Günsel GG, Conlon TM, Wyatt A, Wetzel S, Teupser D, Holdt LM, Ectors F, Boekhoff I, Boehm U, García-Añoveros J, Saftig P, Giera M, Kobold S, Schiller HB, Zierler S, Gudermann T, Wahl-Schott C, Bracher F, Yildirim AÖ, Biel M, Grimm C (2022) Lung emphysema and impaired macrophage elastase clearance in mucolipin 3 deficient mice. Nat Commun 13(1):318. https://doi.org/10.1038/s41467-021-27860-x
76. Abboud RT, Vimalanathan S (2008) Pathogenesis of COPD. Part I. The role of protease-antiprotease imbalance in emphysema. Int J Tuberc Lung Dis 12(4):361–367
77. Voynow JA, Shinbashi M (2021) Neutrophil elastase and chronic lung disease. Biomolecules 11(8):1065. https://doi.org/10.3390/biom11081065
78. O'Sullivan BP, Freedman SD (2009) Cystic fibrosis. Lancet 373(9678):1891–1904. https://doi.org/10.1016/S0140-6736(09)60327-5
79. Twigg MS, Brockbank S, Lowry P, FitzGerald SP, Taggart C, Weldon S (2015) The Role of Serine Proteases and Antiproteases in the Cystic Fibrosis Lung. Mediators Inflamm 2015:293053. https://doi.org/10.1155/2015/293053
80. Gifford AM, Chalmers JD (2014) The role of neutrophils in cystic fibrosis. Curr Opin Hematol 21(1):16–22. https://doi.org/10.1097/MOH.0000000000000009
81. Dittrich AS, Kühbandner I, Gehrig S, Rickert-Zacharias V, Twigg M, Wege S, Taggart CC, Herth F, Schultz C, Mall MA (2018) Elastase activity on sputum neutrophils correlates with severity of lung disease in cystic fibrosis. Eur Respir J 51(3):1701910. https://doi.org/10.1183/13993003.01910-2017
82. Lee W, Downey G (2001) State of the Art Physiological Functions and Role in Acute Lung Injury. Am J Respir Crit Care Med 164:896–904
83. Shirzad M, Hamedi J, Motevaseli E, Modarressi MH (2018) Anti-elastase and anti-collagenase potential of Lactobacilli exopolysaccharides on

human fibroblast. Artif Cells Nanomed Biotechnol 46(sup1):1051–1061. https://doi.org/10.1080/21691401.2018.1443274
84. Godinho MS, Thorpe CT, Greenwald SE, Screen HRC (2021) Elastase treatment of tendon specifically impacts the mechanical properties of the interfascicular matrix. Acta Biomater 123:187–196. https://doi.org/10.1016/j.actbio.2021.01.030

Publisher's Note