Journal of Holistic Integrative Pharmacy 5 (2024) 291–298

Contents lists available at ScienceDirect

Journal of Holistic Integrative Pharmacy

journal homepage: www.keaipublishing.com/en/journals/journal-of-holistic-integrative-pharmacy

Human Epididymis Protein 4 (HE4) as a promising biomarker and therapy target in fibrotic diseases: A review

Huiqun Tian, Li Chen *

Department of Laboratory, The Second People's Hospital of Yichang/The Second People's Hospital of China Three Gorges University, Yichang, 443000, China

ARTICLE INFO

Keywords:
Human Epididymis Protein 4 (HE4)
Pulmonary fibrosis
Renal fibrosis
Myocardial fibrosis
Autoimmune diseases
Liver fibrosis
Anti-fibrotic drugs

ABSTRACT

The treatment of fibrosis faces a significant challenge due to the lack of effective therapies that can reverse established fibrosis. Early detection is vital for intervention, yet distinguishing fibrosis from normal tissue repair is complex. Human Epididymis Protein 4 (HE4), a traditional tumor marker, has been found to be increased in some non-neoplastic conditions, such as fibrosis related diseases. According to properties analysis, HE4 has been characterized as a highly stable cross-class protease inhibitor, which interacts with key fibrotic proteins (such as MMP2 and PRSS family members) and potentially involves in the progression of fibrosis by inhibiting the enzymatic activity of these proteins. Meanwhile, studies indicated that HE4 may be involved in fibrosis through PI3K/AKT, NF-κB, MAPK, and other signaling pathways. Here we summarized the latest research progress of HE4 in pulmonary fibrosis, renal fibrosis, myocardial fibrosis, liver fibrosis, and autoimmune diseases induced fibrosis. As reported in this review, HE4 was closely related to disease severity and prognosis, and also was a promising prognostic evaluation marker and therapeutic intervention target for fibrotic diseases.

1. Introduction

Fibrosis is a continuous pathological change that occurs in response to various forms of tissue damage and plays a role in the development of numerous diseases, including idiopathic pulmonary fibrosis (IPF), chronic obstructive pulmonary disease (COPD), myocardial fibrosis, chronic kidney disease (CKD), and systemic lupus erythematosus (SLE).[1] These kinds of diseases contribute to a high global burden of illness and death. While the Food and Drug Administration (FDA) has approved several antifibrotic medications for managing different fibrotic conditions, current therapeutic approaches are limited to symptom relief and do not adequately address the prevention of fibrogenesis, reversal of established fibrosis, or cure of the disease, particularly in advanced stages.[2] Early detection of fibrosis is crucial for timely intervention, which may prevent disease progression and maintain organ function. Consequently, the discovery of key biomarkers for early diagnosis and monitoring of fibrosis can greatly benefit patients.

Human Epididymis Protein 4 (HE4), an epididymal protein, is a member of whey acidic protein four-disulfide core (WFDC) family with high expression in ovarian serous cancer, lung adenocarcinoma, breast cancer, pancreatic cancer, and transitional cell cancer.[3,4] With the advancement of research, it has been discovered that HE4 is also expressed at moderate or high levels in various non-neoplastic conditions, including renal insufficiency, cystic fibrosis, and heart failure, suggesting that it may be involved in the progression of fibrosis.[5–8] Here, we elucidate the mechanism of HE4 in the process of fibrosis in multiple organs, providing a basis for its clinical application as an indicator of early fibrosis detection and disease monitoring and providing ideas for therapeutic strategies targeting HE4.

2. The pathogenesis of fibrosis

Fibrosis, characterized by excessive deposition of extracellular matrix components resulting from disruption of the normal tissue repair mechanisms, is involved in the advancement of numerous diseases and leads to organ function impairment and eventual failure in severe cases.[9] The molecular process of fibrosis shares many mechanisms with the normal wound healing process and can be broadly categorized into 4 components: blood vessel leakage and extravascular clotting, inflammation and immune response, fibroblast activation and myofibroblast differentiation, and extracellular matrix (ECM)accumulation and reconfiguration (Fig. 1). Damage to tissues from toxins, infections, or other causes, can initiate a complex sequence of events aimed at wound healing.[10] At the outset, epithelial and/or endothelial cells, when damaged, release

* Corresponding author.
E-mail address: chenli18995889811@163.com (L. Chen).

https://doi.org/10.1016/j.jhip.2024.12.001
Received 15 June 2024; Received in revised form 5 December 2024; Accepted 8 December 2024

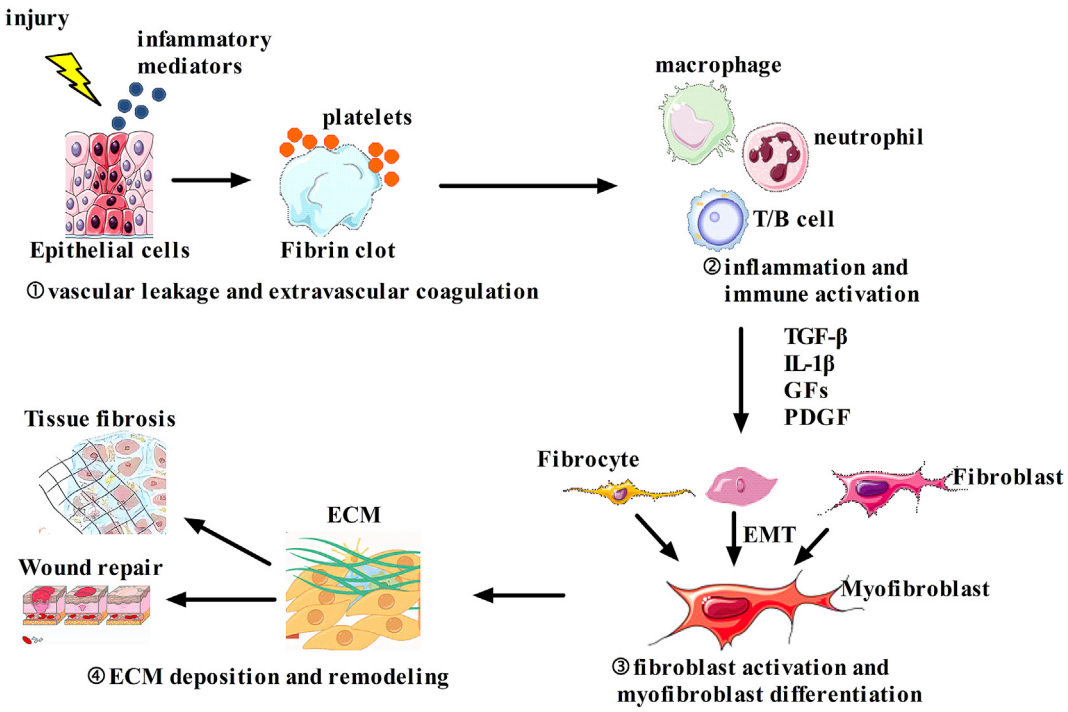

Fig. 1. Presentation of molecular mechanisms of fibrosis.

substances that increase endothelial permeability (i.e., blood vessel leak) and trigger a cascade of events leading to anti-fibrinolytic clotting (i.e., extravascular clotting).[1] Subsequently, an inflammation and immune response phase takes place, characterized by the recruitment, activation, and proliferation of leukocytes like macrophages, neutrophils, and T/B lymphocytes, which are attracted and stimulated by the chemokines and growth factors (GFs) released by epithelial and/or endothelial cells, platelets, and early inflammatory cells.[11] In this process, myofibroblasts and epithelial and/or endothelial cells release matrix metalloproteinases (MMPs), which break down basement membranes and facilitate the recruitment of inflammatory cells to the area of injury.[12] During the acute phase, resident or activated macrophages and neutrophils clear dead cells and harmful substances.[13] As the tissue repair phase commences, macrophages contribute to anti-inflammatory effects as well as the migration and proliferation of fibroblasts and endothelial cells.[11] Concurrently, activated leukocytes release profibrotic cytokines and GFs, such as transforming growth factor beta (TGF-β), interleukin 13 (IL-13), and platelet-derived growth factor (PDGF), which are key in the recruitment, proliferation, and activation of fibroblasts.[14] In a normal wound healing response, activated myofibroblasts would be cleared from the wound site via apoptosis after injury repair.[15] However, in the fibrotic process, myofibroblasts fail to undergo apoptosis and are continuously activated, resulting in excessive deposition of ECM, which ultimately leads to the occurrence of fibrosis.[16] The identification of excessive ECM deposition is the gold standard for the diagnosis of fibrosis. ECM is a non-cellular three-dimensional macromolecular network composed of collagens, proteoglycans/glycosaminoglycans, elastin, fibronectin, laminins, and several other glycoproteins.[17] The dynamic balance between ECM production and degradation is the key to the pathogenesis and development of fibrosis. Under physiological conditions, proteases such as MMPs, contribute to the maintenance of this equilibrium by hydrolyzing ECM components, such as collagen, into small peptides.[18] The dysfunction of proteases impairs the degradation of ECM components in fibrotic diseases, resulting in excessive ECM deposition in fibrotic tissues, which is a common pathological feature in fibrosis.

3. The structure and character of HE4

HE4 is encoded by the Whey-Acidic Four-Disulfide Core domain protein 2 (*WFDC2*) gene that is one of 14 homologous genes on chromosome 20q12–13.1. The *WFDC2* gene spans approximately 12kbp and contains five exons. Full-length HE4 is the result of splicing of exons 1, 2, 4, and 5. Exons 3 and 4 can exist in three forms, two of which can be spliced. The protein encoded by *WFDC2* gene is characterized by its stable disulfide-bonded structure formed by two WFDC domains rich in conserved cysteine residues. The long polypeptide of 124-amino acid is secreted and exists predominantly as disulfide-bonded homotrimer, with each monomer weighing about 14 kDa.[19] N-glycosylation contributes to the protein's stability, which allows HE4 remarkably resilience across a broad pH and temperature range, maintaining its structure even in extreme conditions, such as boiling or the presence of SDS.[20] The expression of HE4 is influenced by factors such as the activation of the NF-κB signaling pathway, tissue-specific expression, hypoxic conditions, estrogen levels, and variability in gene expression.[21,22] These factors collectively determine the expression levels of HE4 across different tissues and pathological states. Based on the research findings from Mary T Galgano et al., the expression of the HE4 gene and protein varies significantly across different tissues under normal conditions.[23] Oligonucleotide microarray analysis revealed that the HE4 gene expression is highest in the normal human trachea and salivary glands, followed by the lung, prostate, pituitary gland, thyroid, and kidney. Meanwhile, tissue microarray and immunohistochemistry methods demonstrated that HE4 protein is expressed in the glandular epithelium of the female reproductive tract, breast, prostate, vas deferens, distal convoluted tubules of the kidney, bronchial epithelium, ductal epithelium of the salivary glands, scattered cells in the anterior pituitary, oxyphilic thyrocytes, lacrimal glands, sweat glands, and colonic epithelium, with varying intensities of expression.[23] Biologically, HE4 has been implicated in various functions, including its role as a cross-class protease inhibitor.[20] It has been shown to inhibit a broad spectrum of serine, cysteine, and aspartyl proteases, suggesting that it may be involved in the regulation of proteolytic activity in biological systems.[20] The inhibition of proteases by

HE4 is essential for its proposed protective role against microbial virulence factors of proteolytic nature, which points towards its significance in innate immunity.[24]

In the context of pathology, elevated HE4 levels have been associated with certain cancers.[25,26] HE4 is highly expressed in ovarian cancers, particularly in serous ovarian carcinomas. Lung adenocarcinomas and breast cancers also exhibit varying levels of HE4 expression, while lung squamous cell carcinomas and prostate cancers show relatively lower levels. Pancreatic and biliary tract cancers, papillary renal cell carcinomas, and certain gastrointestinal cancers demonstrate moderate to high HE4 expression. Malignant mesotheliomas commonly express HE4, and partial expression is observed in thyroid and salivary gland tumors.[23] These variations underscore the potential utility of HE4 as a biomarker in cancer diagnostics and therapeutics. Additionally, there is a growing interest in the relationship between HE4 and fibrosis. Fibrosis, the thickening and scarring of tissues, often involves dysregulated proteolytic activity. Although the direct link between HE4 and fibrosis is not yet fully established, its broad-spectrum protease inhibition could theoretically influence the fibrotic process by modulating the balance between proteases and their inhibitors, thereby affecting extracellular matrix remodeling. In a vitro study, the recombinant HE4 protein showed proteinase inhibitory activity towards trypsin, elastase, and MMP9.[27] Moreover, extracellular HE4 protein increased DNA synthesis and modulated mRNA and protein levels of cell cycle marker proliferating cell nuclear antigen (PCNA) and cell cycle inhibitor p21.[28] All these suggest that HE4 might play a role in the pathogenesis of fibrotic diseases.

4. HE4 involved in fibrosis related diseases

4.1. HE4 and pulmonary fibrosis

4.1.1. Cystic pulmonary fibrosis (CPF)

CPF is an autosomal rare monogenic condition resulting from mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene.[29] More than 2000 mutations have been identified in the *CFTR* gene with the major *p. Phe508del-CFTR* mutation accounting for approximately 80% of all, and other common *CFTR* mutation types including G511D, R553X, G542X, and I1023R. The *CFTR* gene encodes the CFTR protein, which acts as a chloride (Cl-)/bicarbonate (HCO_3-) channel regulating fluid transport across various epithelial surfaces.[29] Elevated expression levels of HE4 are observed in both serum and lung biopsy samples obtained from cystic fibrosis (CF) patients and positively correlated with fibrosis markers.[30] Moreover, overexpression of HE4 enhanced the secretion of inflammatory cytokines and the expression of fibrosis markers. NF-κB and MAPK signaling have been implicated in inflammation and ECM destruction associated with CPF, and the HE4/MAPK/MMPs signaling cascade disrupts normal ECM and promotes fibrosis.[31] Furthermore, studies have confirmed a positive correlation between serum levels of HE4 and overall disease severity as well as pulmonary dysfunction in CF lung patients.[30] Therefore, HE4 can serve as a valuable blood biomarker for diagnosing CPF.

Through a comprehensive analysis of biomarkers in blood, sputum, and bronchoalveolar lavage (BAL) samples of CPF patients to evaluate the pulmonary inflammatory state and treatment effect of CPF patients, the BENE Z team determined that HE4 could be used as a predictive biomarker to monitor the progression of CF lung disease and evaluate the treatment outcome.[32] Following administration of the CFTR modulator ivacaftor (IVA) to individuals with p.Gli551Asp-CFTR mutation, plasma HE4 concentration significantly decreased compared to baseline levels before treatment initiation and continued to decrease within 6 months of treatment.[30] Pulmonary function parameters, specifically forced expiratory volume in 1 s (FEV1; expressed as % predicted), can serve as a valuable clinical tool for evaluating the efficacy of CFTR modulation therapy. Previous studies have demonstrated a negative correlation between plasma HE4 levels and improvements in lung function (delta FEV1) among CF patients undergoing IVA treatment.[30] In individuals with p.Phe508del-CFTR mutation, combination therapy involving CFTR modulators lumacaftor (LUM) and IVA has shown promising results in enhancing lung function, nutritional status, and reducing pulmonary deterioration rates, and the plasma level of HE4 exhibited an inverse relationship with improvement in lung function assessed by percent predicted FEV1.[33]

Recently, the BENE Z group demonstrated that the CFTR-mediated NF-κB pathway played a crucial role in the regulation of HE4 expression in cystic fibrosis bronchial epithelial (CFBE) cells.[34] Dysfunction of CFTR and exposure to external proinflammatory stimuli such as TNF-α through the NF-κB pathway can upregulate HE4 expression and cytokine production, including IL-6, in epithelial cells associated with CF. Conversely, by improving CFTR function using CFTR modulators (LUM/IVA or TEZ/IVA), it is possible to effectively attenuate the proinflammatory response of CFBE cells by inhibiting the NF-κB pathway and preventing excessive expression of IL-6 and HE4. Therefore, this further demonstrates that HE4 can be used as a plasma biomarker to monitor the therapeutic effect of CFTR correctors and enhancers in routine clinical practice and has diagnostic value in monitoring the degree of inflammation reduction in CF patients as a result of restoration of CFTR function.[34]

4.1.2. Interstitial lung disease (ILD)

ILD is a group of pathological diseases characterized by diffuse inflammation and fibrosis in the pulmonary parenchyma and alveoli.[35] It is a broad term encompassing various disease groups that present with clinical symptoms such as dyspnea, widespread infiltrative opacity on chest X-ray, restrictive ventilation impairment, reduced diffusion capacity (DLCO), and hypoxemia.[35] Among them, idiopathic pulmonary fibrosis (IPF) is characterized by unexplained diffuse inflammation of the lower respiratory tract, which invades the alveolar wall and adjacent alveolar space, resulting in alveolar septal thickening and pulmonary fibrosis.[36] Recently, the research team led by TIAN M found that serum HE4 expression was notably elevated in patients with IPF, especially in those with acute exacerbation of IPF (AE-IPF). This finding suggests that serum HE4 can serve as a valuable biomarker for assessing disease severity and predicting poor prognosis in IPF patients.[37] Furthermore, serum HE4 has been further considered as a potential biomarker for connective tissue disease-associated interstitial lung disease (CTD-ILD) and usual interstitial pneumonia associated with connective tissue disease (UIP-CTD)and can be used as a biomarker to assess disease severity and predict prognosis in patients with UIP-CTD.[38] Additionally, there is a substantial increase in both serum and alveolar lavage fluid levels of HE4 among individuals with rheumatoid arthritis-interstitial lung disease (RA-ILD). Therefore, HE4 is expected to be a screening tool and severity assessment marker for RA-ILD patients.[39]

In summary, the present studies show that HE4 can participate in pulmonary fibrosis through MAPK/MMPs and NF-κB signaling (Fig. 2). More studies are needed to elucidate the comprehensive mechanism in the future.

4.2. HE4 and renal fibrosis

Kidney diseases caused by diverse etiologies may develop into renal fibrosis, and ultimately lead to renal failure. Renal fibrosis is a chronic pathological process characterized by tissue remodeling and scar formation within the kidney. During this process, tissue damage, inflammatory response, cytokines, and other factors contribute to the proliferation of myofibroblasts, as well as the synthesis and secretion of ECM. Impaired ECM degradation leads to excessive deposition of ECM, which disrupts the normal architecture of renal tissues and eventually leads to progressive loss of renal function.[40]

In the early stages of renal fibrosis progression, TGF-β1 binds to the type I receptor ALK-5 and the type II receptor BMPR-II on renal tubular epithelial cells, further phosphorylating and activating Smad2/3. The

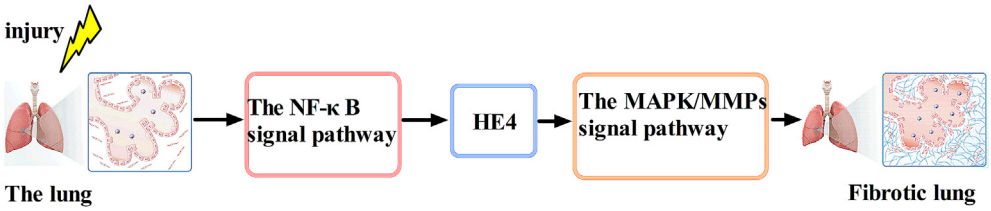

Fig. 2. HE4 promotes fibrosis through MAPK/MMPs and NF-κB pathways in lung.

activated Smad2/3 then complexes with Smad4, translocating to the nucleus and inducing a series of morphological changes such as the formation of fibrous bundles in the renal tubular epithelium, the reduction or disappearance of the apical brush border, the destruction of the basement membrane, and the dissolution of tight junctions between epithelial cells. Subsequently, these cells begin to express mesenchymal markers, transitioning into myofibroblasts with mesenchymal characteristics.[40] Myofibroblasts are widely recognized as the primary effector cells in renal fibrosis. Activated myofibroblasts augment the expression of α-smooth muscle actin (αSMA) and ECM production. Active proteases, including matrix metalloproteinases and serine proteases, stimulate the generation of angiotensin II (Ang II), which promotes fibrosis through binding to AT1 receptors on myofibroblasts and is considered as a pivotal mediator in renal fibrosis.[41] Study has demonstrated that the dynamic equilibrium between the expression of active protease, protease inhibitors such as HE4, and extracellular matrix proteins like collagen and fibronectin may determine whether the outcome of renal injury is reparative healing or fibrosis.[42]

Matrix proteins (biglycan, Bgn; desmin, Des; decorin, Dcn), serine proteases (Prss23 and Prss35), and protease inhibitors(SerpinF1 and Serpina10) have been demonstrated to be associated with fibrosis.[43] In 2013, Le Bleu et al. used a transgenic mouse model expressing fluorescent HE4 protein under the control of the αSMA promoter to identify HE4 as the most up-regulated gene in fibrosis-related myofibroblasts.[44] HE4 is a pan-serine protease inhibitor and inhibitor of matrix metalloproteinases (MMP2 and MMP9), which can promote renal fibrosis by inhibiting the degradation of type I collagen.[45] By inhibiting the serine protease activity of Prss35 and Prss23, HE4 prevents these enzymes from degrading collagen. Administration of neutralizing antibodies against HE4 accelerates type I collagen degradation and inhibits fibrosis.[44] Therefore, HE4 is considered to be a biomarker for predicting renal fibrosis as well as a new therapeutic target.

ZHANG L's team used a mouse model of unilateral ureteral obstruction (Uuo) to investigate the expression and mechanism of HE4 in the pathogenesis of renal fibrosis.[46] The findings revealed that under hypoxia, HIF-1α binded to the promoter region of HE4 to activate its transcription. Subsequently, HE4 expression triggered the activation of the NF-κB pathway through P65 phosphorylation and nuclear translocation. NF-κB upregulates tissue inhibitor of metalloproteinases (TIMPs), which potentially hinder ECM degradation and expedite fibrotic processes by inhibiting MMPs activity.[46] Meanwhile, over-expression of HE4 facilitates the deposition of ECM by up-regulating the expression of COL4A1 and COL1A1, thereby promoting the initiation and progression of renal fibrosis.[44] Clinically acquired nephropathy in patients undergoing coronary intervention is frequently attributed to contrast-induced acute kidney injury (CI-AKI), with the underlying pathological mechanism involving hypoxic injury of proximal renal tubular epithelial cells.[47] A recent study has revealed that dapagliflozin can effectively mitigate oxygen consumption and intracellular hypoxia level by modulating the HIF-1α/HE4/NF-κB pathway, thereby reducing the incidence of CI-AKI.[47] Consequently, it is postulated that renal fibrosis induced by hypoxia occurs via upregulation of HE4 and activation of the HIF-1α/HE4/NF-κB signaling cascade.

Chronic kidney disease (CKD) is a progressive deterioration of renal function, characterized by the development of kidney fibrosis due to excessive accumulation of type I collagen and other extracellular matrix components.[48] The previous study found that the expression of HE4 in renal tubule interstitial positively correlated with the percentage of the renal tubule interstitial fibrosis in patients with CKDs, and serum HE4 significantly increased in female patients with CKD without gynecological tumors.[49] Recently, more and more studies have shown that the level of HE4 is related to the severity and clinical classification of CKD.[50–52]

In summary, current studies have shown that HE4 is involved in the regulation of renal fibrosis by directly or indirectly inhibiting MMPs and other important proteolytic enzymes in fibrosis (Fig. 3). It suggested that HE4 has the potential to serve as a clinical biomarker to assess renal function and predict renal fibrosis. However, further studies are still required to fully understand the mechanism of HE4 in renal fibrosis.

4.3. HE4 and myocardial fibrosis

4.3.1. Pulmonary arterial hypertension (PAH)

PAH is characterized by extensive narrowing and occlusion of the pulmonary vascular system, resulting in increased pulmonary vascular resistance and right ventricular dysfunction.[53] Patients with PAH typically die of right heart failure, which is characterized by right ventricular fibrosis and remodeling. Jin Q et al. found that elevated serum HE4 level in patients with idiopathic pulmonary arterial hypertension (IPAH) was associated with disease severity and could be used as a predictor of clinical deterioration in patients with IPAH and right heart failure, revealing the pathophysiological role of HE4 in tissue fibrosis.[54] Recently, TANG Y's team reported that HE4 was negatively associated with the expression of microRNA-325-3p (miR-325-3p) by constructing a right ventricular fibrosis model and a cardiac fibroblast fibrosis model. And miR-325-3p effectively mitigates right ventricular fibrosis in PAH rats by specifically targeting HE4 and regulating the PI3K/AKT signaling pathway.[55]

4.3.2. Dilated cardiomyopathy (DCM)

DCM is significantly associated with morbidity and mortality resulting from heart failure. Left ventricular pathophysiological remodeling serves as a crucial hallmark of DCM and represents one of the most important indicators of adverse events.[56] The activation of fibroblasts into myofibroblasts, the production of extracellular matrix proteins, cross-linking enzymes, and matrix degradation metalloproteinase inhibitors, and the deposition of extracellular matrix proteins lead to the occurrence and progression of DCM.[57] By investigating the underlying mechanism of HE4-induced fibroblast activation in DCM patients, Yamamoto M's team revealed that HE4 triggered cardiac fibroblast proliferation through extracellular signal-regulated kinase (ERK) signaling, leading to cardiac fibrosis, which is associated with cardiac remodeling and future adverse cardiovascular events.[58] Recent research has revealed that serum HE4 levels significantly correlate with the response to drug treatment in patients with DCM, suggesting that it can be used as a prediction marker of reactive drug treatment, an assessing biomarker for persistent cardiac fibrosis, as well as a new therapeutic target.[59]

4.3.3. Heart failure (HF)

HF is a complex syndrome characterized by impaired cardiac function resulting in insufficient cardiac output to meet the metabolic demands of

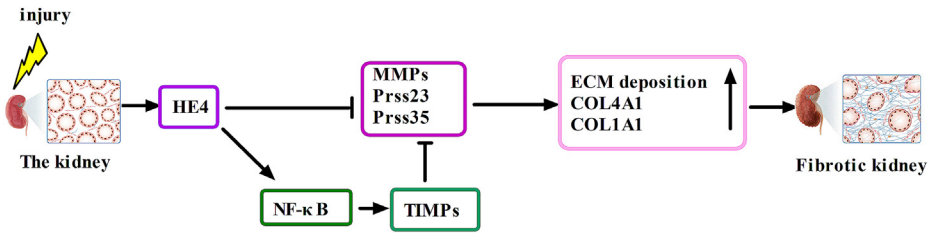

Fig. 3. HE4 promotes fibrosis through directly or indirectly inhibiting MMPs, Prss23 and Prss35 in kidney.

peripheral tissues.[60] Serving as biomarkers for myocardial fibrosis, Galectin-3 (Gal-3) and soluble growth Stimulation expressed gene 2 (ST2) have been incorporated into the American College of Cardiology (ACC)/American Heart Association (AHA)heart failure guidelines.[61] In patients with acute and chronic heart failure, serum HE4 level has been shown to positively correlate with disease severity and prognosis.[62,63] Multivariate modeling was used to explore the correlation between HE4 and fibrosis biomarkers in HF, and it was found that HE4 was closely related to Gal-3, suggesting that HE4 may be involved in organ fibrosis but not specific.[62–64]

Overall, the current studies show that HE4 plays a role in myocardial fibrosis through PI3K/AKT and ERK signaling (Fig. 4). More and more studies have focused on this, and it is believed that more findings about HE4's role in myocardial fibrosis will be discovered in the future.

4.4. HE4 and autoimmune diseases

Autoimmune diseases are a group of diseases characterized by the aberrant targeting of the immune system against the body's own tissues and organs. This immunological dysfunction is often manifested through the production of autoantibodies and the activation of autoreactive T cells, leading to chronic inflammation and tissue damage. A common histopathological feature observed across various autoimmune conditions is the presence of fibrosis, which represents a complex and dynamic process of ECM remodeling.[65] In the context of autoimmune disorders, HE4 has been implicated in Primary Sjögren's Syndrome (pSS), where it correlates with disease activity and systemic involvements, including pulmonary and renal manifestations.[66] This association suggested that HE4 potentially played a role in the clinical stratification of pSS through its expression in affected tissues in the disease. Similarly, in rheumatoid arthritis and its related interstitial lung disease, elevated HE4 levels in serum and bronchoalveolar lavage fluid (BALF) are closely associated with the severity of ILD, indicating that HE4 may be used as a biomarker for screening and assessing the severity of RA-ILD patients.[39] In patients with systemic lupus erythematosus (SLE) and lupus nephritis (LN), the serum level of HE4 is significantly increased in adult-onset LN patients, especially in those with proliferative LN, suggesting that HE4 may be involved in the pathologic of chronic kidney disease caused by SLE.[67] Furthermore, serum HE4 levels in patients with IgG4-Related Disease (IgG4-RD) were significantly higher than those in the control group and were positively correlated with organ damage indicators and fibrosis.[68] In patients with systemic sclerosis (SSc), especially those with SSc-ILD, both serum and BALF HE4 levels are significantly increased, positively correlated with the degree of ILD measured by high-resolution CT (HRCT), and negatively correlated with pulmonary function parameters.[69] Although the mechanism by which HE4 is involved in these diseases has not been clearly defined, it is a potential mediator in the fibrotic process as a protease inhibitor that inhibits the degradation of type I collagen. Although these findings are promising, they have limitations and need to be validated in larger multicenter cohort studies. Future studies are needed to elucidate the specific mechanisms of HE4 in these diseases and establish its utility in clinical diagnosis and prognosis.

4.5. HE4 and liver fibrosis

Liver fibrosis is a pathological repair response to chronic liver injury, which includes damage from viral hepatitis caused by viral infections, alcohol-induced hepatitis from toxic metabolites and oxidative stress produced by ethanol metabolism, non-alcoholic fatty liver disease mediated by lipid metabolism abnormalities, and autoimmune liver diseases characterized by abnormal immune attacks on liver antigens. Regardless of the cause of the fibrosis, it involves the activation of hepatic stellate cells and the excessive deposition of ECM, particularly collagen.[70] Once chronic fibrosis progresses to cirrhosis, it is associated with structural loss and subsequent functional failure, as well as life-threatening complications. Liver biopsy is the gold standard for diagnosing liver fibrosis, but its invasiveness limits its clinical application, making serum marker detection an important auxiliary diagnostic method. The commonly used markers include hyaluronic acid, laminin, procollagen type III, and collagen type IV. However, their diagnostic accuracy and predictive value are limited, and they cannot effectively help establish the grading of liver fibrosis.[71] As a new clinical marker for ovarian cancer, HE4 has been found to play a multifaceted role in the progression of the disease by influencing processes such as cell proliferation, tumor growth, invasion, migration, adhesion, chemoresistance, and steroid biosynthesis. Moreover, HE4 is associated with the activation of various signaling pathways, including the PI3K/AKT pathway, HIF1α, and the ERK/MAPK signaling pathway, the activation of which may be related to the worsening of the tumor and the development of therapeutic resistance.[72] Different from that, HE4 does not show significant expression in liver cancer cells.[23] While, in an experimental study, Hou et al. found that serum HE4 levels were significantly higher in liver fibrosis than those of controls and closely correlated with C–P class.[73] Another study about autoimmune hepatitis also indicated that elevated serum HE4 levels in

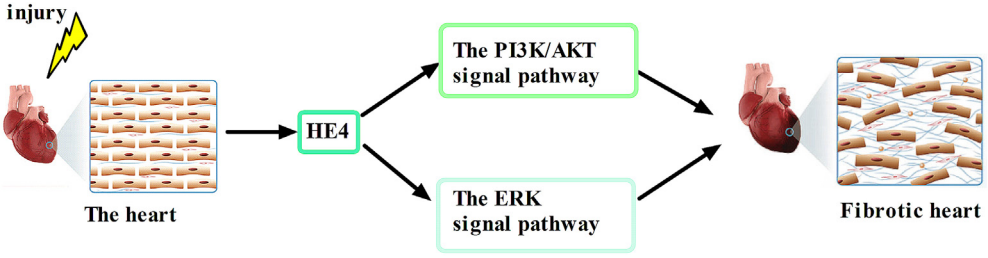

Fig. 4. HE4 promotes fibrosis through PI3K/AKT and ERK pathways in heart.

AIH-LC patients exhibited a strong correlation with the severity of hepatic fibrosis.[74] These studies suggest that HE4 may has the potential to be a marker for the severity of liver fibrosis help to establish the grading of liver fibrosis, although the underlying mechanisms remain elusive.[73,74] However, it is important to note that these studies are retrospective, involve small sample sizes, and have certain limitations. Longitudinal data are necessary for future studies to establish definitive causal relationships.

5. The current situation and challenges of anti-fibrotic drug development

Since the beginning of this century, the search for anti-fibrotic agents has been underway. According to the mechanism of effect, the anti-fibrotic drugs under development are mainly classified into three classes: 1) drugs targeting core signaling pathways in fibrosis; 2) drugs targeting effectors in fibrosis; 3) drugs targeting other contributors to fibrosis (such as lipid mediator inhibitors, modulators of the cellular stress response, modulators of the immune system and inflammation, and drugs of use in cardiovascular/metabolic affections). The three classes of drugs contain numerous inhibitors and many of them have entered the clinical trial stage. Among them, the types of drugs targeting core pathways are the most numerous, with details shown in Table 1.

However, for more than two decades, only two drugs (Pirfenidone and Nintedanib) have been authorized against two specific pathologies of the respiratory tract, and other drugs are still in clinical trials or have not shown the expected efficacy. According to the literature review, there are three main reasons for drug development failure: 1) Lack of efficacy, possibly due to the pharmacological target being "other fibrosis contributing factors"; 2) Inhibition by a single mechanism often fails to achieve the desired effect; 3) Safety: Many drugs are limited to clinical use due to toxic effects, and safety issues often arise for drugs involving the inhibition of core signaling pathways.[96] In addition, the dynamic balance of multiple factors in the process of tissue injury is widely involved, and different tissues may have different reactions to different drugs. These factors together make anti fibrosis drugs face enormous challenges.

6. Discussion and conclusion

Fibrosis is a key point for many chronic diseases to develop into irreversible tissue damage and loss of function. The reversal of fibrosis is crucial to intervene in the development of chronic diseases and improve the prognosis. The identification of novel biomarkers that facilitate the early detection of fibrosis is essential for enabling timely clinical intervention.

HE4, recognized as a biomarker for malignancy traditionally, has been found to be increased in the serum of patients with certain non-neoplastic chronic diseases. Moreover, HE4 has been characterized as a highly stable cross-class protease inhibitor according to properties analysis, suggesting a significant role in human physiology. Especially, HE4 suppresses the activity of key proteins such as matrix metalloproteinase 2 (MMP2), protease serine 23 (PRSS23), and protease serine 35 (PRSS35), specifically inhibits their capacity to degrade type I collagen, which is pivotal for fibrotic.[46] Therefore, it can be inferred that HE4 is essential to the process of fibrosis.

This review synthesizes the most recent advancements in research concerning the role of HE4 in pulmonary, renal, myocardial, liver, and a series of autoimmune diseases. Although most of these studies have just focused on correlating serum HE4 levels with disease severity, prognostic outcomes, and specific disease indicators in clinical cohorts, and few studies have preliminary tried to explore its mechanism underlying HE4's involvement in fibrosis, the potential clinical utility of HE4 as a biomarker in various organ fibrosis is promising. Nevertheless, further in-depth mechanistic investigations, especially the direct involvement of HE4 in fibrosis-related diseases, are imperative to substantiate these

Table 1
The main category of anti-fibrotic drugs and related mechanisms.

Drug Category	Mechanisms in fibrosis	Reference
Drugs targeting core signaling pathways in fibrosis		
TGFβ inhibitors	TGF-βpromotes fibrosis through several mechanisms leading to activation and recruitment of myofibroblasts and to parenchymal cellular death, or directly induces fibrosis.	75,76
CTGF inhibitors	CTGF participates in the differentiation, proliferation and chemotaxis of myofibroblasts, and synergize with other profibrotic mediators, such as TGF-β or Wnt.	77
PDGF inhibitors	PDGF bind to tyrosine kinase PDGF receptors (PDGFR), whose activation promote the mitogenic andchemoattractant effects of mesenchymal cells, and induce transitional processes in other cell types to generate myofibroblasts.	78
Pirfenidone and derivates	Pirfenidone inhibit the action of fibrogenic growth factors and limit fibroblast proliferation, myofibroblast differentiation and ECM production.	79
Multiple tyrosine kinase inhibitors	This group agent inhibits VEGFR, FGFR, PDGFR, and Src kinases and can reduce fibrosis by inhibiting theepithelial-mesenchymal transition, the proliferation of fibroblasts and their activation into myofibroblasts.	80–82
Jak inhibitors	The JAK pathway regulates the differentiation, proliferation, death of cells and inflammation.	83
Integrin αvβ inhibitors	Integrins sense both chemical and physical properties of ECM, are responsible for mechano-transduced pathways contributing to fibrosis, and mediate the release and activation of the latent ECM-embedded TGF-β.	84
Rock inhibitors	Rock family is essential for actin cytoskeleton remodeling	85
Amphiregulin (AREG) inhibitors	Amphiregulin plays a critical role in restoring tissue integrity following infection or injury by modulating the immune response	86
WNT and hedgehog pathway inhibitors	These interconnected signals might regulate myofibroblast formation and proliferation and contribute to fibrosis.	87
miRNA-29 mimic	MiRNA-29 suppresses the expression of a wide range of ECM proteins and profibrotic signaling molecules.	88
Drugs targeting effectors in fibrosis		
HSP47 inhibitors	HSP47 specifically interacts with procollagens, and is crucial for different stages of collagen synthesis.	89
TG2 inhibitors	TG2 mediates collagen cross-linking and the activation of matrix-bound latent TGF-β in fibrosis.	90
LOX/LOXL inhibitors	LOX/LOXL catalyzes the conversion of lysine into aldehyde residue in ECM proteins, which allows subsequent collagen cross linking.	91
MMP7 inhibitors	In theory, Matrix metalloproteinases (MMPs) limit fibrosis though degrading ECM. However, several evidences show that MMPs promote fibrotic responses to injury by modulating many mediators and processes involved in its progression.	92
Drugs targeting other contributors to fibrosis		
Lipid mediator inhibitors	During chronic inflammation, the persisted up-regulation of LPA induces the proliferation, migration and activation of fibroblasts.S1P modulates the activation of myofibroblast, and is part of a complex crosstalk system of profibrotic mediators that includes TGF-β.	93,94
Modulators of the cellular stress response	Oxidative stress (OS) promotes fibrosis through causing cell damage, promoting inflammation and inducing fibroblasts directly activated.	95

findings from multiple perspectives, thereby enhancing the predictive and potentially therapeutic applications of HE4 in fibrotic conditions.

In view of the current status of anti-fibrotic drug research, HE4 is a potential therapeutic target with the following advantages: 1) A protease inhibitor: HE4 participates in the control of ECM balance by regulating protease activity, which is critical in the fibrosis process.[46] 2) Participate in the regulation of inflammatory response: HE4 plays an important role in the inflammatory process, which is a common trigger of fibrosis.[97] 3) Potential biomarkers: The expression level of HE4 in fibrotic tissues is correlated with disease activity and can be used as a biomarker for disease progression, which is helpful for diagnosis and monitoring treatment effect. 4) HE4 is involved in multiple signaling pathways related to fibrosis. It seems that the simultaneous blockade of several pathways provides a greater beneficial effect. In fact, Pirfenidone and Nintedanib, two currently marketed antifibrotic agents, are characterized by multifaceted effects on central pathways in fibrosis.[96] 5) HE4 is expressed at a relative low level under normal physiological conditions and only in some tissues (http://www.humanproteomemap.org/batch.php), which means it may have a good safety to be a therapy target. Notably, it is important to acknowledge the challenge in distinguishing the fibrotic process from the normal tissue repair mechanisms, which complicates early identification of fibrosis. The expression profile and mechanistic role of HE4 in this context also warrant urgent exploration to facilitate a deeper understanding. Another point to note is that the lack of organ/-tissue specificity is also a challenge in the clinical application of HE4. Overall, as the role of HE4 in the treatment of fibrosis has not been fully studied, it provides an area of research to explore new therapeutic approaches.

CRediT authorship contribution statement

Huiqun Tian: Writing – review & editing. **Li Chen:** Writing – review & editing.

Declaration of competing interest

There are no conflicts of interest.

Acknowledgements

This research was supported by the medical scientific research projects of Yichang (A22-22-051).

References

1. Zhang Y, Huang W, Jiao H, et al. Molecular imaging of fibrosis in benign diseases: an overview of the state of the art. *Pharmaceuticals*. 2024;17(3):296.
2. Xing L, Chang X, Shen L, et al. Progress in drug delivery system for fibrosis therapy. *Asian J Pharm Sci*. 2021;16(1):47–61.
3. Muinao Thingreila, Boruah Hari Prasanna Deka, Pal Mintu. Multi-biomarker panel signature as the key to diagnosis of ovarian cancer. *Heliyon*. 2019;5(12):e02826.
4. Kumarasamy C, Madhav MR, Sabarimurugan S, et al. Diagnostic and prognostic role of HE4 expression in multiple carcinomas: a protocol for systematic review and meta-analysis. *Medicine (Baltim)*. 2019;98(28):e15336.
5. Wang L, Sun Y, Cai X, et al. The diagnostic value of human epididymis protein 4 as a novel biomarker in patients with renal dysfunction. *Int Urol Nephrol*. 2018;50(11):2043–2048.
6. Béla Nagy Jr, Nagy Béla, Fila Libor, et al. Human Epididymis Protein 4: a novel serum inflammatory biomarker in cystic fibrosis. *Chest*. 2016;150(3):661–672.
7. Rudolf A de Boer, Cao Qi, Postmus Douwe, et al. The WAP four-disulfide core domain protein HE4: a novel biomarker for heart failure. *JACC Heart Fail*. 2013;1(2):164–169.
8. Nishiyama N, Masuo M, Nukui Y, et al. Human epididymis protein 4 is a new biomarker to predict the prognosis of progressive fibrosing interstitial lung disease. *Respir Investig*. 2021;59(1):90–98.
9. Zhao X, Kwan Jennifer Yin Yee, Yip Kenneth, et al. Targeting metabolic dysregulation for fibrosis therapy. *Nat Rev Drug Discov*. 2020;19(1):57–75.
10. Wynn Thomas A, Ramalingam Thirumalai R. Mechanisms of fibrosis: therapeutic translation for fibrotic disease. *Nat Med*. 2012;18(9):1028–1240.
11. Henderson Neil C, Rieder Florian, Wynn Thomas A. Fibrosis: from mechanisms to medicines. *Nature*. 2020;587(7835):555–566.
12. Talbott Heather E, Mascharak Shamik, Griffin Michelle, et al. Wound healing, fibroblast heterogeneity, and fibrosis. *Cell Stem Cell*. 2022;29(8):1161–1180.
13. Sousa Ana Beatriz, Aguas Artur P, Barbosa Mario A, et al. Immunomodulatory biomaterial-based wound dressings advance the healing of chronic wounds via regulating macrophage behavior. *Regen Biomater*. 2022;9:rbac065.
14. Hassanshahi Alireza, Moradzad Mohammad, Ghalamkari Saman, et al. Macrophage-mediated inflammation in skin wound healing. *Cells*. 2022;11(19):2953.
15. Mascharak Shamik, Talbott Heather E, Januszyk Michael, et al. Multi-omic analysis reveals divergent molecular events in scarring and regenerative wound healing. *Cell Stem Cell*. 2022;29(2):315–327.
16. Hinz Boris, Lagares David. Evasion of apoptosis by myofibroblasts: a hallmark of fibrotic diseases. *Nat Rev Rheumatol*. 2020;16(1):11–31.
17. Lena Schön Johanna, Groß Victoria Elisabeth, Post Willem Berend, et al. The adhesion GPCR and PCP component flamingo (FMI-1) alters body size and regulates the composition of the extracellular matrix. *Matrix Biol*. 2024;128:1–10.
18. Luangmonkong Theerut, Parichatikanond Warisara, Olinga Peter. Targeting collagen homeostasis for the treatment of liver fibrosis: opportunities and challenges. *Biochem Pharmacol*. 2023;215:115740.
19. Desbène Cédric, Maiga Ramatou Yéya, Gaillard Olivier. Immunoanalytical characteristics of HE4 protein. *Ann Biol Clin*. 2018;76(2):225–233.
20. Chhikara Nirmal, Saraswat Mayank, Kumar Tomar Anil, et al. Human Epididymis Protein-4 (HE-4): a novel cross-class protease inhibitor. *PLoS One*. 2012;7(11):e47672.
21. Jiang SW, Chen H, Dowdy Sean, et al. HE4 transcription- and splice variants-specific expression in endometrial cancer and correlation with patient survival. *Int J Mol Sci*. 2013;14(11):22655–22677.
22. Kim Kyukwang, Khazan Negar, McDowell Jamie L, et al. The NF-κB-HE4 axis: a novel regulator of HE4 secretion in ovarian cancer. *PLoS One*. 2024;19(12):e0314564.
23. Mary T Galgano, Hampton Garret M, Frierson Jr Henry F. Comprehensive analysis of HE4 expression in normal and malignant human tissues. *Mod Pathol*. 2006;19(6):847–853.
24. Schirinzi Annalisa, Pia Cazzolla Angela, Lovero Roberto, et al. New insights in laboratory testing for COVID-19 patients: looking for the role and predictive value of human epididymis secretory protein 4 (HE4) and the innate immunity of the oral cavity and respiratory tract. *Microorganisms*. 2020;8(11):1718.
25. Li J, Li Y, Huo L, et al. Detection of serum HE4 levels contributes to the diagnosis of lung cancer. *Oncol Lett*. 2023;25(6):255.
26. Chudecka-Głaz Anita, Strojna Aleksandra, Michalczyk Kaja, et al. Evaluation of He4 use in the diagnosis of ovarian cancer: first and second recurrence, and an analysis of HE4 concentration during second- and third-line chemotherapy. *Diagnostics*. 2023;13(3):452.
27. Hua L, Liu Y, Zhen S, et al. Expression and biochemical characterization of recombinant human epididymis protein 4. *Protein Expr Purif*. 2014;102:52–62.
28. Guo F, Li J, Qi Y, et al. HE4 overexpression decreases pancreatic cancer Capan-1 cell sensitivity to paclitaxel via cell cycle regulation. *Cancer Cell Int*. 2020;20:163.
29. Myer Heather, Chupita Sarah, Jnah Amy. Cystic fibrosis: back to the basics. *Neonatal Netw*. 2023;42(1):23–30.
30. Béla Nagy Jr, Zsolt Bene, Fejes Zsolt. Human epididymis protein 4 (HE4) levels inversely correlate with lung function improvement (delta FEV1) in cystic fibrosis patients receiving ivacaftor treatment. *J Cyst Fibros*. 2019;18(2):271–277.
31. Wang J, Zhao H, Xu F, et al. Human epididymis protein 4 (HE4) protects against cystic pulmonary fibrosis associated-inflammation through inhibition of NF-κB and MAPK singnaling. *Genes Genomics*. 2019;41(9):1045–1053.
32. Bene Zsolt, Fejes Zsolt, Macek Jr Milan, et al. Laboratory biomarkers for lung disease severity and progression in cystic fibrosis. *Clin Chim Acta*. 2020;508:277–286.
33. Pócsi Marianna, Fejes Zsolt, Bene Zsolt, et al. Human epididymis protein 4 (HE4) plasma concentration inversely correlates with the improvement of cystic fibrosis lung disease in p.Phe508del-CFTR homozygous cases treated with the CFTR modulator lumacaftor/ivacaftor combination. *J Cyst Fibros*. 2023;22(6):1085–1092.
34. Bene Zsolt, Fejes Zsolt, Szanto Tibor Gabor, et al. Enhanced expression of Human Epididymis Protein 4 (HE4) reflecting pro-inflammatory status is regulated by CFTR in cystic fibrosis bronchial epithelial cells. *Front Pharmacol*. 2021;12:592184.
35. Wijsenbeek Marlies, Suzuki Atsushi, Maher Toby M. Interstitial lung diseases. *Lancet*. 2022;400(10354):769–786.
36. Rajesh Rishi, Atallah Reham, Bärnthaler Thomas. Dysregulation of metabolic pathways in pulmonary fibrosis. *Pharmacol Ther*. 2023;246:108436.
37. Tian M, Meng K, Gao Y, et al. Elevated serum human epididymis protein 4 is associated with disease severity and worse survival in idiopathic pulmonary fibrosis: a cohort study. *Ann Transl Med*. 2022;10(18):992.
38. Meng K, Tian M, Gui X, et al. Human epididymis protein 4 is associated with severity and poor prognosis of connective tissue disease-associated interstitial lung disease with usual interstitial pneumonia pattern. *Int Immunopharm*. 2022;108:108704.
39. Lin T, Xu S, Wang Y, et al. Human epididymis protein 4 as a new diagnostic biomarker for rheumatoid arthritis-associated interstitial lung disease. *Clin Exp Rheumatol*. 2022;40(11):2167–2174.
40. Wang J, Li J, Zhang X, et al. Molecular mechanisms of histone deacetylases and inhibitors in renal fibrosis progression. *Front Mol Biosci*. 2022;9:986405.
41. Xu Z, Zou C, Yu W, et al. Inhibition of STAT3 activation mediated by toll-like receptor 4 attenuates angiotensin II-induced renal fibrosis and dysfunction. *Br J Pharmacol*. 2019;176(14):2627–2641.
42. Lindquist Jonathan A, Mertens Peter R. Myofibroblasts, regeneration or renal fibrosis—is there a decisive hint? *Nephrol Dial Transplant*. 2013;28(11):2678–2681.
43. Oriano Martina, Amati Francesco, Gramegna Andrea, et al. Protease-antiprotease imbalance in branchiectasis. *Int J Mol Sci*. 2021;22(11):5996.

44. LeBleu Valerie S, Teng Yingqi, O'Connell Joyce T, et al. Identification of human epididymis protein-4 as a fibroblast-derived mediator of fibrosis. *Nat Med*. 2013;19(2):227–231.
45. Chen P, Yang Q, Li X, et al. Potential association between elevated serum human epididymis protein 4 and renal fibrosis: a systemic review and meta-analysis. *Medicine (Baltim)*. 2017;96(36):e7824.
46. Zhang L, Liu L, Bai M, et al. Hypoxia-induced HE4 in tubular epithelial cells promotes extracellular matrix accumulation and renal fibrosis via NF-κB. *Faseb J*. 2020;34(2):2554–2567.
47. Huang X, Guo X, Yan G, et al. Dapagliflozin attenuates contrast-induced acute kidney injury by regulating the HIF-1α/HE4/NF-κB pathway. *J Cardiovasc Pharmacol*. 2022;79(6):904–913.
48. Mavrogeorgis Emmanouil, Mischak Harald, Latosinska Agnieszka, et al. Collagen-derived peptides in CKD: a link to fibrosis. *Toxins*. 2021;14(1):10.
49. Liu L, Ning X, Bai M, et al. Prognostic and diagnostic value of HE4 expression in patients with chronic kidney diseases. *Int J Clin Exp Pathol*. 2016;9(8):7930–7940.
50. Miao Q, Cai B, Niu Q, et al. Changes in lung cancer-related serum tumor markers in patients with chronic kidney disease and determination of upper reference limit. *Front Oncol*. 2022;12:1072531.
51. Yan S, Lin Y, Tian X. Significantly elevated serum human epididymis protein-4 in chronic kidney disease patients without ovarian cancer: a large-scale retrospective study. *J Clin Lab Anal*. 2023;37(4):e24847.
52. Song J, Chen L, Yuan Z, et al. Elevation of serum human epididymis protein 4 (HE4) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) as predicting factors for the occurrence of acute kidney injury on chronic kidney disease: a single-center retrospective self-control study. *Front Pharmacol*. 2023;14:1269311.
53. Barragan-Martinez Maria-Del-Pilar, Cueto-Robledo Guillermo, Roldan-Valadez Ernesto, et al. A brief review on gender differences in Mexican-Mestizo patients with Pulmonary Arterial Hypertension (PAH) at a tertiary-level hospital. *Curr Probl Cardiol*. 2022;47(9):101275.
54. Jin Q, Tang Y, Liu Z, et al. Serum human epididymis protein 4 level as a predictor of clinical worsening in idiopathic pulmonary arterial hypertension: a pilot study. *BMC Cardiovasc Disord*. 2020;20(1):175.
55. Tang Y, Huo X, Liu J, et al. MicroRNA-325-3p targets human epididymis protein 4 to relieve right ventricular fibrosis in rats with pulmonary arterial hypertension. *Cardiovasc Ther*. 2022:4382999.
56. Amemiya Kisaki, Matsuyama Taka-Aki, Ishibashi-Ueda Hatsue, et al. Can right ventricular endomyocardial biopsy predict left ventricular fibrosis beforehand in dilated cardiomyopathy? *ESC Heart Fail*. 2024;11(2):1001–1008.
57. Zhang J, Cheng L, Li Z, et al. Immune cells and related cytokines in dilated cardiomyopathy. *Biomed Pharmacother*. 2024;171:116159.
58. Yamamoto Masahiro, Hanatani Shinsuke, Araki Satoshi, et al. HE4 predicts progressive fibrosis and cardiovascular events in patients with dilated cardiomyopathy. *J Am Heart Assoc*. 2021;10(15):e021069.
59. Qiu Q, Peng J, Liu D. Evaluation of the baseline HE4 Level for predicting drug responsiveness in patients with dilated cardiomyopathy. *Chin Heart J*. 2023;35(3):310–315.
60. D'Amario Domenico, Laborante Renzo, Delvinioti Agni, et al. GENERATOR HEART FAILURE DataMart: an integrated framework for heart failure research. *Front Cardiovasc Med*. 2023;10:1104699.
61. Le Goff Caroline. Galectin-3 and suppression of tumorigenicity 2: two emerging cardiac biomarkers that may be predictors of cardiac fibrosis development in sport. *Exp Physiol*. 2023;108(10):1257–1258.
62. Piek Arnold, Meijers Wouter C, Schroten Nicolas F, et al. HE4 serum levels are associated with heart failure severity in patients with chronic heart failure. *J Card Fail*. 2017;23(1):12–19.
63. Tang Y, Hu Z, Liu Z, et al. HE4 serum levels are associated with poor prognosis in patients with acute heart failure combined with chronic kidney disease. *Int J Gen Med*. 2024;17:1273–1280.
64. Piek Arnold, Du Weijie, de Boer Rudolf A, et al. Novel heart failure biomarkers: why do we fail to exploit their potential? *Crit Rev Clin Lab Sci*. 2018;55(4):246–263.
65. Tang Y, Li L. The application of nanovaccines in autoimmune diseases. *Int J Nanomed*. 2024;19:367–388.
66. Chen J, Sun F, Bao H, et al. Elevated serum human epididymis protein 4 is associated with disease activity and systemic involvements in primary Sjögren's Syndrome. *Front Immunol*. 2021;12:670642.
67. Li L, Xu H, Le Y, et al. Elevated serum levels of human epididymis protein 4 in adult patients with proliferative lupus nephritis. *Front Immunol*. 2023;14:1179986.
68. Yan S, Peng Y, Wu Z, et al. Human epididymis protein 4 (HE4) is a novel biomarker for fibrosis in IgG4-related disease and can predict poor prognosis. *RMD Open*. 2022;8(2):e002521.
69. Zhang M, Zhang L, Linning E, et al. Increased levels of HE4 (WFDC2) in systemic sclerosis: a novel biomarker reflecting interstitial lung disease severity? *Ther Adv Chronic Dis*. 2020;11:2040622320956420.
70. Roehlen Natascha, Crouchet Emilie, Baumert Thomas F. Liver fibrosis: mechanistic concepts and therapeutic perspectives. *Cells*. 2020;9(4):875.
71. Sumanth Nallagangula Krishna, Kurpad Nagaraj Shashidhar, Venkataswamy Lakshmaiah, et al. Liver fibrosis: a compilation on the biomarkers status and their significance during disease progression. *Future Sci OA*. 2017;4(1):FSO250.
72. James Nicole E, Chichester Clinton, Ribeiro Jennifer R. Beyond the biomarker: understanding the diverse roles of Human Epididymis Protein 4 in the Pathogenesis of epithelial ovarian cancer. *Front Oncol*. 2018;8:124.
73. Hou Y, Li F, Chen J, et al. Clinical significance of serum human epididymis protein 4 in liver fibrosis: an experimental study. *Medicine (Baltim)*. 2020;99(48):e23428.
74. Yu Z, Nian C, Sun W, et al. Elevated serum HE4 levels as a novel biomarker of disease severity and hepatic fibrosis in autoimmune hepatitis. *Clin Chim Acta*. 2024;559:119682.
75. Hermina Györfi Andrea, Matei Alexandru-Emil, Distler Jörg HW. Targeting TGF-β signaling for the treatment of fibrosis. *Matrix Biol*. 2018;68–69:8–27.
76. Sonnylal Sonali, Denton Christopher P, Zheng Bing, et al. Postnatal induction of transforming growth factor β signaling in fibroblasts of mice recapitulates clinical, histologic, and biochemical features of scleroderma. *Arthritis Rheum*. 2007;56(1):334–344.
77. Qiu Y, Que Y, Ding Z, et al. Drugs targeting CTGF in the treatment of pulmonary fibrosis. *J Cell Mol Med*. 2024;28(10):e18448.
78. Ai JY, Liu CF, Zhang W, et al. Current status of drugs targeting PDGF/PDGFR. *Drug Discov Today*. 2024;23:103989.
79. Ruwanpura Saleela M, Thomas Belinda J, Bardin Philip G. Pirfenidone: molecular mechanisms and potential clinical applications in lung disease. *Am J Respir Cell Mol Biol*. 2020;62(4):413–422.
80. Lehmann Mareike, Lara Buhl, Alsafadi Hani N, et al. Differential effects of Nintedanib and Pirfenidone on lung alveolar epithelial cell function in ex vivo murine and human lung tissue cultures of pulmonary fibrosis. *Respir Res*. 2018;19(1):175, 15.
81. Ahangari Farida, Becker Christine, Foster Daniel G, et al. Saracatinib, a selective src kinase inhibitor, blocks fibrotic responses in preclinical models of pulmonary fibrosis. *Am J Respir Crit Care Med*. 2022;206(12):1463–1479.
82. Ezelarab Hend AA, El-Hafeez Amer Ali Abd, Ali Taha FS, et al. New 2-oxoindole derivatives as multiple PDGFRα/ß and VEGFR-2 tyrosine kinase inhibitors. *Bioorg Chem*. 2024;145:107234.
83. Siba P Raychaudhuri, Raychaudhuri Smriti K. JAK inhibitor: introduction. *Indian J Dermatol, Venereol Leprol*. 2023;89(5):688–690.
84. Maldonado Horacio, Hagood James S. Cooperative signaling between integrins and growth factor receptors in fibrosis. *J Mol Med (Berl)*. 2021;99(2):213–224.
85. Xie Y, Yue L, Shi Y, et al. Application and study of ROCK inhibitors in pulmonary fibrosis: recent developments and future perspectives. *J Med Chem*. 2023;66(7):4342–4360.
86. Zhang MY, Fang S, Gao H, et al. A critical role of AREG for bleomycin-induced skin fibrosis. *Cell Biosci*. 2021;11(1):40.
87. Ren D, Luo J, Li Y, et al. Saikosaponin B2 attenuates kidney fibrosis via inhibiting the Hedgehog pathway. *Phytomedicine*. 2020;67:153163.
88. Deng Z, He Y, Yang X, et al. MicroRNA-29: a crucial player in fibrotic disease. *Mol Diagn Ther*. 2017;21(3):285–294.
89. Sakamoto Noriho, Okuno Daisuke, Tokito Takatomo. HSP47: a therapeutic target in pulmonary fibrosis. *Biomedicines*. 2023;11(9):2387.
90. Tatsukawa Hideki, Hitomi Kiyotaka. Role of transglutaminase 2 in cell death, survival, and fibrosis. *Cells*. 2021;10(7):1842.
91. Chen W, Yang A, Jia J, et al. Lysyl oxidase (LOX) family members: rationale and their potential as therapeutic targets for liver fibrosis. *Hepatology*. 2020;72(2):729–741.
92. Craig Vanessa J, Zhang Li, Hagood James S, et al. Matrix metalloproteinases as therapeutic targets for idiopathic pulmonary fibrosis. *Am J Respir Cell Mol Biol*. 2015;53(5):585–600.
93. Donati Chiara, Cencetti Francesca, Bernacchioni Caterina, et al. Role of sphingosine 1-phosphate signalling in tissue fibrosis. *Cell Signal*. 2021;78:109861.
94. Magkrioti Christiana, Galaris Apostolos, Kanellopoulou Paraskevi, et al. Autotaxin and chronic inflammatory diseases. *J Autoimmun*. 2019;104:102327.
95. Otoupalova Eva, Smith Sam, Cheng Guangjie, et al. Oxidative stress in pulmonary fibrosis. *Compr Physiol*. 2020;10(2):509–547.
96. Fuster-Martínez Isabel, Calatayud Sara. The current landscape of antifibrotic therapy across different organs: a systematic approach. *Pharmacol Res*. 2024;205:107245.
97. Lai X, Kang M, Chen Y, et al. Elevated serum level of human epididymal protein 4 (HE4) predicts poor prognosis in the critically ill with sepsis: a prospective observational cohort study. *Clin Biochem*. 2022;109–110:79–85.