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HE4: a potential diagnostic biomarker in autoimmune diseases

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Abstract

Human epididymal protein 4 (HE4), also known as protein four-disulfide core domain 2, is a secretory protein that is highly expressed in epithelial ovarian cancer. HE4 has higher specificity and sensitivity than traditional biomarkers in ovarian cancer, making it an effective marker for monitoring the progression of ovarian cancer. Given the similarities between the pathological processes in cancer and autoimmune diseases (ADs), namely overactivation of immune cells and involvement of inflammatory responses, patients with ADs may have an increased risk of cancer. Accumulating evidence suggests that HE4 is strongly associated with ADs such as systemic lupus erythematosus, Sjögren's syndrome, rheumatoid arthritis-associated interstitial lung disease, and immunoglobulin A nephropathy. Several studies have reported that HE4 reflects disease severity and has excellent potential as a biomarker for the diagnosis and progression monitoring of ADs. In this article, we provide a detailed overview of the research on the biological function of HE4 in ADs and provide insights into whether HE4 has the potential to serve as a candidate biomarker for chronic inflammatory ADs.

Key words: *HE4, autoimmune disease, biomarker.*

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Introduction

Autoimmune diseases (ADs) are a group of inflammatory connective tissue disorders caused by immune system dysfunction leading to faulty immune responses to autoantigens. They are typically characterized by the production and deposition of immune complexes [1]. ADs affect approximately 10% of the global population [2]. Despite tremendous efforts over the past few decades to gain insight into these chronic diseases, their disease mechanisms are poorly understood and their prevalence continues to increase, particularly in high-income, industrialized countries, such as the United States [3, 4]. In fact, autoimmunity is the third most common cause of death in Western countries [5]. In China, drastic changes in lifestyle and improvements in disease diagnosis have led to ADs gradually becoming a significant public health issue requiring substantial medical resources [6]. From 1990 to 2021, the incidence and prevalence of several ADs have trended upward in China, including type 1 diabetes, inflammatory bowel disease, psoriasis, and rheumatoid arthritis [6]. A population-based cohort study conducted in 23 provincial regions of China revealed an increasing number of patients with systemic lupus erythematosus (SLE), particularly among women aged 30-49 years [7]. In 2017, the standardized incidence of SLE among Chinese women was 26.41 per 100,000, and the standardized prevalence was 94.16 per 100,000 individuals, both of which are significantly higher than those for men [7]. Since the course of most ADs is relatively prolonged, patients not only bear long-term harm to their physical and mental well-being but also require substantial financial support [8].

The occurrence and development of ADs involves multiple factors, and increasing evidence indicates that both genetic susceptibility and environmental factors contribute to the onset and aggravation of ADs [9-11]. Unfortunately, the precise pathogenesis of many ADs has yet to be determined.

Significant progress has been made in the diagnosis and treatment of ADs; however, owing to the insidiousness of these heterogeneous diseases, subtle lesions may not be promptly detected. Therefore, it is necessary to develop diagnostic tools for ADs with high sensitivity and specificity for clinical use. Biomarkers have proven to be suitable choices for diagnosis. Since ADs cause varying degrees of inflammation and/or organ damage, changes in the composition and concentration of biomarkers in human blood could reflect disease progression [12, 13]. Although imaging and histopathological examinations remain the preferred diagnostic tools for most diseases,

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biomarkers have many advantages, including enhanced disease monitoring and applications in precision medicine. In addition, because of their ease of operation and cost-effectiveness, serum biomarkers can be used for testing in healthy individuals.

Interleukin 6 (IL-6), as a pleiotropic cytokine secreted by lymphocytes and monocytes, is involved in many pathophysiological processes, including inflammation, vascular permeability, tissue regeneration, and metabolism [14]. Elevated levels of IL-6 in serum and tissues are a proven hallmark of SLE and rheumatoid arthritis (RA), as well as an effective indicator of disease activity [15, 16]. C-reactive protein (CRP) is an acute-phase protein widely used in clinical practice as a systemic biomarker for diagnosing and monitoring inflammatory conditions and tissue damage [17]. When infection or inflammation occurs, hepatocytes produce and release CRP in large quantities, and its concentration can increase dramatically by 1000-fold or more [18]. In addition, abnormally elevated CRP levels have been incorporated into the 2010 classification criteria for rheumatoid arthritis established by the American College of Rheumatology/European League Against Rheumatism [19]. Critically, ADs can lead to the production of autoantibodies targeting specific tissues, and high-titer autoantibodies are often used for clinical diagnosis of ADs and treatment decision-making [20, 21]. There are many types of autoantibodies; among the most important are antinuclear antibodies (ANA), which are not only recognized as laboratory biomarkers that could be used for the screening and diagnosis of ADs but have also been included in the enrollment criteria for SLE [22, 23].

Human epididymis protein 4 (HE4) has received increasing attention for its potential as a biomarker for monitoring ovarian cancer [24]. Compared to traditional clinical biomarkers, HE4 has shown superior performance in detecting early stage ovarian cancer [25]. The potential use of HE4 as a biomarker for ovarian cancer was first reported by Hellstrom *et al*. in 2003 [26]. A prospective multi-center clinical trial in 2008 confirmed that HE4 detection contributed to the diagnosis of ovarian tumors [27]. In the same year, the U.S. Food and Drug Administration approved HE4 as a blood biomarker for monitoring ovarian cancer recurrence or progression [28]. The usefulness of monitoring HE4 is being explored for other diseases, such as lung cancer, endometrial cancer, and renal fibrosis. Evidence suggests that HE4 has remarkable potential as a diagnostic marker for early cancer detection and may be a novel therapeutic target [29-31].

Recently, a few studies have investigated the potential association between HE4 and ADs. Data have shown that immune dysregulation and chronic inflammation occur not only in ADs but are also strongly associated with tumors [32]. In fact, a dynamic and bidirectional relationship exists between cancer and autoimmunity [33]. Some studies have shown that patients with autoimmune rheumatic diseases have a higher risk of cancer than the general population, mainly because they not only require ongoing immunosuppressive therapy but also suffer from significant tissue damage and are more susceptible to infections by oncogenic viruses [34-36]. Current evidence indicates that specific tumor biomarkers relevant to cancer might have clinical potential for detecting ADs. Although research on HE4 and autoimmunity is sparse, many studies have observed abnormal expression of HE4 in ADs. Therefore, this review summarizes the biological functions and molecular mechanisms of HE4, presents recent studies on HE4 in autoimmunity, discusses the potential of HE4 as a diagnostic and prognostic biomarker in ADs, and provides future perspectives for AD detection strategies.

Literature review method

A comprehensive search was conducted of the public literature databases PubMed and Web of Science for all eligible articles published until April 30, 2025. The search terms included “HE4” and “autoimmune disease”. In addition, the references cited in the retrieved literature were searched for additional relevant studies.

Basic research on HE4

Molecular structure and biological functions of HE4

Human epididymal protein 4, or HE4, was discovered in 1991 by Kirchhoff *et al*. in a study of cloned cDNA sequences containing the complete coding region of a novel human single-copy gene [37]. The HE4 polypeptide contains a signal peptide and abundant cysteine residues and exhibits characteristics typical of a secreted protein [37]. It is a bidomain member of the “four-disulfide core” protein family and is commonly used as a protease inhibitor. HE4 is expressed in a tissue-specific manner, and HE4 mRNA is localized in the epithelial cells at the distal portion of the epididymal duct and vas deferens, suggesting that it may be involved in sperm maturation [37].

Expression of HE4

Similar to most other biomarkers, HE4 is expressed in both malignant and normal human tissues [38]. Galgano *et al*. performed a comprehensive study on the mRNA and protein expression levels of HE4 in various normal and malignant adult tissues using oligonucleotide and tissue microarrays [39]. This study revealed significant HE4 immunoreactivity in multiple normal tissues, including the mammary, prostate, vas deferens, and bronchial epithelia as well as in the salivary glands, anterior pituitary gland, and female reproductive tract. Malignant tumors positive for HE4 included ovarian tumors, lung adenocarcinoma, colorectal cancer, breast cancer, and transitional

cell carcinoma [39]. The oligonucleotide microarray results were consistent with the immunohistochemistry findings; among the 43 tested normal human tissues, HE4 expression was highest in the trachea and salivary glands. Among the tested malignant tissues, ovarian cancer showed much higher expression levels than other tumor tissues [39]. Given the elevated expression of HE4 in ovarian tumors, it is now recognized as a clinically valuable diagnostic marker and is widely used to monitor the progression of ovarian cancer and the efficacy of prognosis [40].

Serum reference values of HE4

In healthy individuals, serum HE4 levels vary according to sex and age [41]. Age is an essential factor in determining HE4 levels; as age increases, the concentration of HE4 increases sharply [42]. A large-scale Nordic study of 1,591 participants established age-dependent reference values for HE4; a multivariate analysis determined that the reference limit for HE4 in normal women aged 18 years was 51.5 pmol/l, while the threshold increases to 69.7 pmol/l in women aged 82 years [41]. Similar results were observed in another study of 300 healthy multi-ethnic Asian women, with HE4 concentrations increasing with age. Notably, there was a statistically significant difference in serum HE4 levels between two different ethnic groups, Malaysians and Indians [43].

For detecting epithelial ovarian cancer, the recommended serum HE4 thresholds are 70 pmol/l for premenopausal women and 140 pmol/l for postmenopausal women [44]. Although elevated serum HE4 levels can be detected in other cancers, further study and verification are required to establish diagnostic cutoff values [45].

Mechanisms of HE4

The precise mechanisms of HE4 are still being explored; however, previous molecular and cellular studies have provided critical information. Lu *et al*. observed that the expression of HE4 significantly regulates the adhesion, migration, and abnormal proliferation of ovarian cancer cells [46]. Overexpression of HE4 activates the EGFR-dependent MAPK signaling pathway, promoting the phosphorylation of ERK 1/2 and thus increasing the invasion and growth of cancer cells [46]. Zhu *et al*. reported similar findings [47]. Interestingly, the MAPK/ERK signaling pathway is also important in ADs [48]. Wang *et al*. showed that inhibition of the MAPK/ERK signaling pathway could eliminate reactive oxygen species and reduce the secretion of inflammatory cytokines, thereby ameliorating cellular oxidative stress and inflammation, ultimately leading to effective inhibition of keratinocyte proliferation in immune-mediated inflammatory skin diseases [49]. Although we only have indirect evidence suggesting that HE4 regulates the occurrence of ADs by modulating the MAPK/ERK signaling pathway, this could be a focus in future research.

NF-κB, a factor in a critical inflammatory pathway, has been shown to be involved in various diseases [50]. Previous reports have indicated that abnormal activation of NF-κB is a key link between autoimmunity and cancer [51]. Activated NF-κB significantly upregulates the expression of pro-inflammatory cytokines; in turn, the increased levels of key pro-inflammatory cytokines could further stimulate the NF-κB signaling pathway [52]. This positive feedback mechanism creates a favorable environment for tumors and inflammation-related diseases. Recently, Kim *et al*. confirmed that overexpression of HE4 in ovarian cancer is controlled by the NF-κB pathway and is associated with tumor progression and poor clinical outcomes [53]. Moreover, Zhang *et al*. reported that HE4 expression was positively correlated with renal interstitial fibrosis [54]. Increased expression of HE4 activates the NF-κB signaling pathway, which significantly inhibits the activity of matrix metalloproteinase 2 (MMP-2), thereby preventing degradation of the extracellular matrix and inducing renal fibrosis [54]. Renal fibrosis is the leading cause of death in patients with lupus nephritis, a complication of SLE [55].

Application of HE4 in autoimmune diseases

Systemic lupus erythematosus

Systemic lupus erythematosus is a complicated connective tissue disease involving multiple systems that poses a serious health threat, particularly in women [56]. The clinical manifestations of SLE range from mild rashes to symmetrical joint pain and even devastating multi-organ failure. The prevalence of SLE varies considerably across geographic regions and ethnicities [57]. According to an epidemiological survey, the overall incidence of SLE in a U.S. population receiving health insurance is approximately 49 per 100,000, with a prevalence of approximately 366.6 per 100,000 [58]. In contrast, the prevalence in the UK population was much lower, at approximately 97.04 per 100,000 individuals [59]. Despite the significant medical and economic burdens on affected individuals and society, the specific pathogenesis of SLE remains unclear, thus necessitating in-depth research to determine the pathogenesis and reduce these burdens.

A case-control study from southwest China reported that HE4 was highly correlated with disease activity and systemic involvement in SLE [60]. Compared to age- and sex-matched healthy participants, individuals with pediatric-onset SLE (pSLE) had markedly elevated serum levels of HE4, and a receiver operating characteristic (ROC) analysis indicated that 56.9 pmol/l is the optimal diagnostic threshold [60]. Patients with disease involvement in certain organs or tissues, including the hematological, pulmonary, and cardiovascular systems, had higher HE4 levels than those without these clinical manifestations [60].

Notably, the increase in HE4 levels in patients with renal involvement was directly proportional to the inflammatory parameters that reflect kidney damage, such as the neutrophil-to-lymphocyte ratio and the levels of uric acid, creatinine, and blood urea nitrogen [60].

Anti-dsDNA antibodies and complement components C3 and C4 are widely used as traditional biomarkers for SLE, primarily for disease monitoring and to assess clinical activity [61]. However, these indicators exhibit relatively low sensitivity and specificity for monitoring specific organ involvement, particularly kidney damage. In contrast, HE4 has shown significant diagnostic and monitoring performance as a candidate biomarker in SLE [60, 62].

Lupus nephritis

Lupus nephritis (LN) is a form of lupus with severe kidney damage that occurs in 50%–70% of patients with SLE [63]. LN has an insidious onset with fluctuating conditions and is a key cause of death in patients with SLE [64]. Therefore, it is necessary to identify biomarkers that closely reflect LN progression.

Ren *et al*. conducted a prospective study of 74 patients with newly diagnosed SLE who were regularly followed up. During follow-up, 44 patients developed LN, and their serum HE4 levels gradually increased [65]. Multivariate regression analysis showed that HE4 was an independent risk factor for the occurrence of LN; specifically, a 2.7-fold increase in serum HE4 levels led to a 16.8-fold increase in the risk of LN [65]. These data suggest that HE4 is a valid predictor of LN, and a serum HE4 value of 64.8 pmol/l is the optimal cut-off. A similar finding was reported in a retrospective single-center study; serum HE4 levels were markedly elevated in adult patients with proliferative LN, particularly in those with grade IV active LN and active/chronic (A/C) lesions [66].

Anti-dsDNA and anti-C1q antibodies are disease guideline-recommended surveillance tools for LN that can effectively distinguish LN from other types of nephritis [67, 68]. However, because LN is histologically divided into six pathological types, the available clinical biomarkers may be associated only with a specific type, which would have detrimental effects on timely disease detection and treatment, as diagnosis will be delayed [69]. Using HE4 as a testing tool may overcome the deficiencies of traditional markers in disease management of LN [66].

Sjögren's syndrome

Sjögren's syndrome (SS) is a lymphoproliferative disorder with autoimmune features that can be divided into two types: primary and secondary [70]. Lymphocytic infiltration of the lacrimal and salivary glands is a typical feature of SS, which leads to extreme dryness of the mouth, eyes, and other mucosal surfaces [71]. As the severity and clinical manifestations of SS vary, accurate diagnosis and effective treatment remain challenging. Therefore, greater efforts are needed to explore this proliferative AD to identify clinically useful biomarkers [70].

Chen *et al*. reported that HE4 has diagnostic value for clinical stratification of primary SS (pSS) and can be used to determine lung and renal involvement and disease severity [72]. In their study, serum HE4 levels were much higher in patients with pSS than in healthy controls (103.65 pmol/l vs. 46.52 pmol/l), and serum HE4 levels were more significantly elevated in patients with moderately to highly active disease than in patients with inactive disease [72]. Additionally, ROC analysis revealed 69.50 pmol/l HE as the optimal cutoff value to effectively distinguish between active and non-active pSS [72].

Autoantibodies against Ro/SSA and La/SSB are clinical diagnostic markers that are included in the classification criteria for pSS [73, 74]. However, a large-sample retrospective study reported that anti-SSA and anti-SSB antibodies may not have diagnostic or monitoring efficacy in approximately one-third of patients with pSS. Therefore, this subset of patients could develop severe interstitial lung disease [75]. Although the association between HE4 and SS is still at the exploratory stage and addition evidence is required to confirm the suitability of HE4 as a biomarker for SS, HE4 may serve as a useful monitoring tool for patients with pSS and specific organ involvement, including lung and kidney damage.

Rheumatoid arthritis-associated interstitial lung disease

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by symmetric arthritis that leads to irreversible damage to the cartilage and bone, with an overall incidence of 0.5-1% [76]. Although this disease primarily affects the joints, it is also considered a syndrome because it can affect multiple systems, such as the pulmonary system, including the interstitium, airways, and pleura [76]. Rheumatoid arthritis-associated interstitial lung disease (RA-ILD), which is the most common clinical extra-articular manifestation of RA, occurs in 10-40% of patients [77, 78]. Interstitial lung disease is a significant cause of death in patients with RA, with a median survival of only 4.9 years and a five-year survival rate of approximately 59.7% [79, 80]. Therefore, there is an urgent need for improved diagnosis and monitoring of disease progression to improve treatment efficacy and patient prognosis.

In a cross-sectional study of HE4 levels in patients with RA, peripheral blood and bronchoalveolar lavage fluid samples were collected from 102 patients, and HE4 levels were measured using an electrochemiluminescence immunoassay [81]. Patients diagnosed with RA-ILD had significantly higher concentrations of HE4 in the serum and bronchoalveolar lavage fluid than both healthy individuals and patients with RA without RA-ILD. More

importantly, HE4 levels were positively correlated with high-resolution computed tomography fibrosis scores and negatively correlated with pulmonary function [81]. ROC analysis identified HE4 concentrations of 111.1 pmol/l in serum and 595 pmol/l in bronchoalveolar lavage fluid as valid cutoff values for predicting ILD in patients with RA [81]. More importantly, HE4 levels were positively correlated with high-resolution computed tomography fibrosis scores and were negatively correlated with parameters of pulmonary function [81]. A recent meta-analysis revealed a surprising finding: HE4 was inversely correlated with the pulmonary function parameter forced vital capacity, which can be used to assess the severity of RA-ILD [82]. Both of these innovative studies suggested that HE4 has potential for clinical screening and assessment of disease activity in RA-ILD, which will facilitate timely detection and treatment.

Peripheral blood biomarkers that can accurately identify RA-ILD for use in clinical practice are lacking. Although several studies have indicated that matrix metalloproteinase-7 (MMP-7) and Krebs von den Lungen-6 (KL-6) are closely associated with the risk of RA-ILD [83], their specificity is insufficient, and further research is needed to determine their utility for dynamic monitoring of disease progression [84, 85]. Unlike MMP-7 and KL-6, HE4 may perform better, and with continued in-depth investigation, it may prove to be useful for later clinical diagnoses and making treatment decisions.

Other autoimmune diseases

Immunoglobulin A nephropathy (IgAN) is a form of glomerulonephritis in which the deposition of immunoglobulins causes inflammatory responses that ultimately damage the glomeruli and lead to the loss of kidney function [86]. Invasive renal biopsy remains the gold standard for the clinical diagnosis of IgAN [87]. However, owing to the challenges in detecting small lesions in early stage disease, there is an urgent need for less invasive diagnostics and more effective treatments to delay disease progression and enhance patients' quality of life. A recent retrospective study showed that HE4 levels were substantially higher in patients with IgAN than in normal controls (116.43 ±03.61 pmol/l vs. 35.57 ±9.33 pmol/l), and HE4 levels were strongly associated with glomerulosclerosis [88]. Based on the Youden index, Luo *et al*. established a HE4 level of 45.15 pmol/l as the credibility threshold to distinguish patients with IgAN [88]. Another key finding was a significant positive correlation between higher serum HE4 levels and the severity of renal pathological damage in IgA patients. These findings suggest that HE4 may be a non-invasive biomarker for predicting the occurrence of IgAN and assessing disease severity with high specificity and sensitivity.

Autoimmune hepatitis (AIH) is an inflammatory disease predominantly characterized by liver damage that can occur at any age and is more common in women [89, 90]. The diagnostic criteria for AIH include serum biochemical and histological changes in the liver, such as elevated levels of aminotransferase and immunoglobulin G, and positivity for serum ANA [91]. Notably, in both the chronic latent and acute-onset early stages of AIH, diagnosis remains a considerable challenge because of the lack of specific serological findings. Yu *et al*. used a chemiluminescence assay to measure and compare serum HE4 levels in patients with AIH and healthy subjects, and found a significant difference between the groups, as serum HE4 levels in patients with AIH (73.50 pmol/l) were generally higher than those in healthy controls (48.7 pmol/l), and the HE4 levels of patients with liver cirrhosis (98.60 pmol/l) were the highest [92]. The study identified 82.34 pmol/l HE4 as a threshold for assessing the risk of liver cirrhosis in patients with AIH. Elevated serum HE4 levels (≥ 82.34 pmol/l) were not only independent risk factors for predicting the occurrence of liver cirrhosis but also closely associated with adverse outcomes. Overall, these findings support the use of HE4 as a clinically valuable diagnostic tool for assessing the severity of AIH.

Discussion

Over the past decade, studies focusing on the relationship between HE4 and autoimmunity have indicated that HE4 has potential as a novel biomarker for the diagnosis of ADs and assessment of disease severity. In existing reports, we found relevant evidence indicating that elevated levels of HE4 are associated with the risk of developing ADs. However, it is important to note that according to previous studies, the incidence of autoimmune diseases differs among different races and between sexes. HE4 expression also shows a certain degree of variation. In addition, the diagnostic thresholds established for HE4 vary among ADs. Furthermore, the impact of inconsistencies in the HE4 detection methods used, such as enzyme-linked immunosorbent assays and electro-chemiluminescent immunoassays, cannot be overlooked. Another important point is that sample size is a critical aspect of a study, and none of the identified studies exceeded 200 cases; the sample sized ranged from 74 to 182. However, if the sample size is insufficient, selection bias may occur, which affects the validity and reliability of the research findings. Table 1 provides detailed information from these studies [60,65,66,72,81,88,92].

The molecular mechanisms and pathophysiological role in the disease of any novel biomarker approved for clinical practice should be clearly understood and specific. Unfortunately, little is known about how HE4 functions in autoimmunity, and no current hypotheses can explain its role in detail. Some studies suggest that inflammation and cell proliferation-related pathways involved in the occurrence of cancer are also important for ADs (Table 2) [93-104]. Abnormal activation of the NF-κB signaling pathway can lead to an accumulation of pro-inflammatory

Table 1. Application of HE4 in autoimmune diseases

Author, year (citation)	Type of disease	Detection method	Testing instrument	Number of cases	Cut-off value for HE4	AUC	95% CI	Specificity	Sensitivity
Liu *et al*., 2024 [60]	Pediatric-onset systemic lupus erythematosus	Chemiluminescent immunoassay	Abbott ARCHITECT i2000SR	137	56.9 pmol/l	0.607	0.533-0.681	Not applicable	Not applicable
Ren *et al*., 2018 [65]	Lupus nephritis	Enzyme-linked immunosorbent assay	Not applicable	74	64.8 pmol/l	0.714	0.597-0.831	53.30%	81.8%
Li *et al*., 2023 [66]	Lupus nephritis	Chemiluminescent immunoassay	Abbott ARCHITECT i2000SR	182	57.1 pmol/l	0.865	0.793-0.916	85.40%	70.50%
Chen *et al*., 2021 [72]	Sjögren's syndrome	Electrochemiluminescence	Roche Cobas E601	109	69.50 pmol/l	0.739	0.645-0.833	65.30%	80.0%
Lin *et al*. 2022 [81]	Rheumatoid arthritis-associated interstitial lung disease	Electrochemiluminescence immunoassay	Roche Diagnostics GmbH	102	111.1 pmol/l	0.782	0.683-0.881	86%	65.9%
Luo *et al*., 2024 [88]	Immunoglobulin A nephropathy	Chemiluminescent immunoassay	Abbott Alinity	89	47.15 pmol/l	0.929	0.882-0.975	88.3%	89.9%
Yu *et al*., 2024 [92]	Autoimmune hepatitis	Electrochemiluminescent immunoassay	Roche Diagnostics GmbH	109	82.34 pmol/l	0.782	0.697-0.867	79.07%	69.70%

cytokines and chemokines, stimulate abnormal survival of reactive B lymphocytes, and produce various autoantibodies associated with ADs [105]. Several critical signaling pathways that regulate cell proliferation have also been associated with the development of ADs. Excessive growth and differentiation of T cells, B cells, and other immune cells leads to the loss of self-antigen recognition, resulting in erroneous attacks, destruction of immune tolerance, severe tissue damage, and persistent, chronic inflammation [106]. Ultimately, these events generate a favorable micro-environment for the development of autoimmunity. Although there is no direct and reliable evidence indicating that the molecular mechanism of HE4 in ADs involves regulation of the aforementioned signaling pathways, this is a direction worth exploring in future research.

Compared to traditional biomarkers, HE4 not only possesses relatively high sensitivity and specificity but is also closely associated with disease progression and even poor patient prognosis. For certain ADs, the lack of effective markers for screening or diagnostics may lead to delays in timely diagnosis and treatment. HE4 has the potential to address such deficiencies in clinical monitoring. Furthermore, HE4 may be capable of detecting organ involvement in patients with ADs earlier, which would facilitate timely diagnoses and assist clinicians in making treatment decisions. Thus, HE4 remains a valuable research area in ADs.

However, despite these promising findings, several issues remain unresolved. First, most existing studies had relatively small sample sizes and were conducted exclusively in China. Therefore, the evidence supporting HE4 as a diagnostic marker for ADs must be further confirmed. Since the incidence and prevalence of ADs vary across ethnicities and geographic regions, validation using large cohorts with different populations is necessary. Second, the role of HE4 in the pathogenesis of ADs remains unclear. The molecular mechanisms involving HE4 in AD must be determined before HE4 can be applied in clinical practice. Third, given the different characteristics of populations and disease states, it is necessary to establish reference intervals or cutoff values based on the HE4 levels in various ADs. Fourth, the impact of the differences in HE4 detection methods must be addressed. Standardized processing should be prioritized to ensure the repeatability, consistency, and reliability of the results.

In summary, HE4 has potential as a convenient, cost-effective biomarker and novel diagnostic indicator, providing new possibilities for the diagnosis of ADs. Future research should include as many cases as possible of different races and from different regions as well as multi-center and prospective validation studies with long-term follow-up. In addition, detailed investigations on the molecular mechanism of HE4, its application in conjunction with traditional biomarkers, and the integration of multidisciplinary approaches are needed to maximize the clinical application value of HE4 in autoimmunity.

Table 2. Signaling pathways associated with autoimmune diseases

Author, year (citation)	Signaling pathway	Type of disease
Ilchovska *et al*., 2021 [93]	NF-kB	Rheumatoid arthritis
Zubair *et al*., 2013 [94]	NF-kB	Systemic lupus erythematosus
Chen *et al*., 2019 [95]	NF-kB	Inflammatory bowel disease
Mc *et al*., 2013 [96]	NF-kB	Multiple sclerosis
Mavropoulos *et al*., 2013 [97]	p38 MAPK	Psoriasis
Zhao *et al*., 2022 [98]	p38 MAPK	Immunoglobulin A nephropathy
Wang *et al*., 2016 [99]	ERK/MAPK	Systemic lupus erythematosus
Ten *et al*., 2021 [100]	ERK/MAPK	Multiple sclerosis
Liu *et al*., 2020 [101]	PI3K/AKT	Rheumatoid arthritis
Zhang *et al*., 2024 [102]	PI3K/AKT	Autoimmune prostatitis
He *et a*l., 2024 [103]	PI3K/AKT	Autoimmune thyroiditis
Zhao *et al*., 2020 [104]	PI3K/AKT	Lupus nephritis

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