REVIEW ARTICLE

Peptidyl Arginine Deiminase 4: APromising Therapeutic Target in Rheumatoid Arthritis and Other Diseases

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Abstract

The present review offers comprehensive data on the roles of Peptidyl Arginine Deiminase 4 (PAD4) in various diseases, including rheumatoid arthritis (RA). PAD4, an enzyme responsible for converting arginine to citrulline, has been linked to the development of chronic inflammation. The current study is a narrative review to determine whether PAD4 is a potential therapeutic target for RA and other illnesses. We searched PubMed and Google Scholar for studies published before 2024. The potential therapeutic utility of PAD4 in various diseases such as cancer, miscarriage, multiple sclerosis, retinopathy, and RA is emphasized in the article. This paper explores the advancements in the development of small-molecule inhibitors and monoclonal antibodies targeting PAD4. It stresses the importance of conducting additional research to resolve specificity, safety, and sustained effectiveness concerns. The review emphasizes the need for a comprehensive approach to completely comprehend the varied attributes and potential therapeutic applications of PAD4.

Key words: Anti-cyclic citrullinated peptide antibodies, chronic inflammation, peptidyl arginine deiminase 4, rheumatoid arthritis

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune condition where the body attacks its joints, causing inflammation and other symptoms. Genetic and environmental factors like tobacco use can trigger RA, which primarily targets synovial joints, starting with small peripheral joints and often spreading to larger ones.[1] Chronic inflammation leads to joint damage, cartilage breakdown, and bone erosion. Early RA lasts less than six months, while established RA persists longer. If untreated, RA worsens over time, increasing the risk of illness and death.[1]

Environmental factors, combined with genetic predisposition, can trigger RA in certain individuals. RA patients often have antibodies targeting citrullinated proteins, first identified in 1964 through the antiperinuclear factor. Later, in 1979, anti-keratin antibodies were found, and in the 1990s, these antibodies were shown to specifically target citrullinated filaggrin.[2,3] These antibodies, called anti-cyclic citrullinated peptide antibodies (ACPAs), can be identified using cyclic citrullinated peptide (CCP) in enzyme-linked immunosorbent assays (ELISA).[4] Citrulline is produced by the enzyme peptidyl arginine deiminase (PAD) at sites of tissue damage and inflammation, such as the lungs in smokers.[1]

Patients with a genetic predisposition may produce anti-modified protein antibodies in response to altered proteins.

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The enzyme PAD, particularly its isoforms PAD2 and PAD4, is linked to RA by modifying arginine into citrulline. Anti-PAD4 antibodies, specific to RA and associated with ACPA, can be detected in RA patients.[5] In gingivitis, the bacterium *Porphyromonas gingivalis* triggers inflammation and leukocyte proliferation, which produces PAD4.[1] Peptidyl arginine deiminase 4 (PAD4) is a crucial enzyme for gene expression and the conversion of arginine to citrulline. It governs processes such as cell death, immune defense, and cell differentiation. Dysregulation of PAD4 can lead to various diseases, making it a potential target for the treatment of RA.[6]

Peptidyl arginine deaminase 4 (PAD4) is an enzyme that alters proteins by converting arginine to citrulline and aids in the formation of neutrophil extracellular traps (NETs). PAD4 is crucial in the development of cardiovascular diseases, autoimmune disorders, and cancers, making it a potential target for detection and treatment.[7,8] Studies have linked PAD expression and citrullination to conditions such as RA, prion disease, psoriasis, Alzheimer's, multiple sclerosis, cancer, and diabetes.[9] Jones *et al*. highlight the growing recognition of citrullination's role in RA and other diseases, with PADs, particularly PAD4, identified as promising therapeutic targets.[10]

The current study is a narrative review to determine whether peptidyl arginine deiminase 4 (PAD4) is a promising therapeutic target in RA and other disorders. We used Google Scholar and the Trip database to search for studies about PAD 4 and its role in RA and other diseases. Furthermore, for more reference coverage, we went through the references to reviews. The keywords we used were the role of PAD in RA, the role of PAD in other diseases, how PADs work, PAD4 in RA, and PAD4 inhibitors.

BIOCHEMICAL AND MOLECULAR ASPECTS OF PAD4

Histones are crucial components of chromatin, influencing DNA binding and gene transcription. Posttranslational modifications (PTMs) such as phosphorylation, methylation, acetylation, ubiquitination, and citrullination alter histone function.[11-13] Citrullination, facilitated by PAD enzymes, converts peptidyl-arginine to peptidyl-citrulline and is associated with diseases such as RA, Alzheimer's, multiple sclerosis, lupus, Parkinson's, and cancer.[14,15] The human PAD family includes five calcium-dependent isozymes (PADs 1-4 and 6), sharing approximately 50% sequence similarity. PADs are distributed across various tissues: PAD1 in the epidermis and uterus, PAD2 in muscle, brain, and secretory glands, PAD3 in hair follicles and keratinocytes, PAD4 in granulocytes and cancers, and PAD6 in oocytes and embryos.[14] Although all PADs are cytoplasmic, only PAD4 is known to deiminate histones, with PAD2 potentially playing a role. Recent research indicates that PADs are also present in granules, mitochondria, and nuclei.[16,17] In addition to histones, PADs can target proteins such as fibrinogen, filaggrin, and actin, which are citrullinated in RA.[18]

PAD4 IN RA

In 1998, researchers identified the involvement of the PAD enzyme in RA. Autoantibodies in RA patients recognize citrulline as a crucial autoantigen. The CCP assay detected these as anti-CCPs, or ACPAs, which are vital serological markers for RA diagnosis.[19] ACPAs can be detected years before the onset of RA and are associated with preclinical inflammation, severe joint disease, and rapid radiographic progression.[20] As ACPAs gained significance in RA research, PAD2, and PAD4 were pinpointed as major drivers of pathogenic citrullination. Studies in mice and humans found these enzymes in hematopoietic cells (granulocytes, monocytes, and macrophages) within the RA synovium, while other PAD isoforms were absent. Deleting or inhibiting PAD2 or PAD4 in mouse models significantly reduced RA severity, underscoring their role in disease progression.[21-27]

PAD2 and PAD4 produce citrullinated proteins, such as fibrinogen, vimentin, and α-enolase, which serve as autoantigens in RA.[28,29] However, the precise role of each PAD enzyme in contributing to the RA citrullinome remains uncertain. While PAD2 and PAD4 preferentially citrullinate different but partially overlapping substrates, neither enzyme alone can generate all the citrullinated antigens observed in RA.[30-32] These studies reveal distinct citrullination patterns, which may lead to autoantigens with varying immunogenicity. ACPAs in the blood of RA patients bind to citrullinated fibrinogen from both PAD2 and PAD4 at low concentrations, but at high concentrations, they favor PAD4-citrullinated fibrinogen.

PAD enzymes are pivotal in the aberrant citrullination observed in RA, leading to autoimmunity against citrullinated antigens. This dual role makes PAD enzymes both contributors to and targets of the autoimmune response.[5] Disrupted citrullination can cause the immune system to attack PAD enzymes, potentially initiating a cycle that leads to key RA symptoms: generation of citrullinated autoantigens, release of pro-inflammatory cytokines, and joint damage. Some RA patients have autoantibodies targeting PAD4, PAD2, or both, which correlate with different clinical outcomes, indicating that anti-PAD antibodies could serve as predictive biomarkers.[5] Incorporating these antibodies alongside ACPAs, RF, and other markers could aid in better categorizing RA patients. Anti-PAD4 antibodies can either intensify or mitigate joint damage in RA, depending on their specific epitopes.[28,33] Ferucci *et al*. found that anti-PAD4 antibodies are more prevalent in RA patients than in first-degree relatives without RA, suggesting they emerge earlier than anti-CCP antibodies in RA development.[34] Studies also associate high levels of PAD, citrullination, and related diseases, including RA, prion

disease, psoriasis, Alzheimer's, multiple sclerosis, cancer, and others.[9]

ROLE OF PAD4 IN DIFFERENT DISEASES

Studies have explored PAD's role in glioblastoma multiforme (GBM), where PAD pathways influence the formation and release of extracellular vesicles (EVs) in GBM and other cancers.[35] These EVs facilitate tumor growth, angiogenesis, and invasion. A PAD3 inhibitor reduced GBM cell invasion and altered EVs in LN229 cells, though PAD4 and PAD2 inhibitors were more effective in LN18 cells.[35]

Elevated PAD4 expression is associated with chemotherapy resistance in hepatocellular carcinoma (HCC) patients undergoing transcatheter arterial chemoembolization post-surgery. The overproduction of PAD4 in HCC cells induces autophagy, a protective mechanism against cytotoxicity, leading to chemotherapy resistance.[36,37] Exosomes and microvesicles are crucial in intercellular communication, transporting molecules such as cytokines, growth factors, and miRNAs that influence processes such as differentiation, migration, and angiogenesis.[36-39] In addition, PAD2 and PAD4 have been shown to translocate to the nucleus in response to TNF overexpression.[40-42]

Renal insufficiency affects about 50% of cancer patients at diagnosis, leading to reduced effectiveness of anticancer treatments due to lowered medication dosages. In mice models, kidney function showed decreased creatinine clearance and increased urine protein levels. Electron microscopy revealed reversible changes, such as increased mesangial cells, but no permanent damage such as fibrosis or necrosis. Treatment with DNase I or PAD4 inhibitors restored renal function by eliminating NETs. PAD4 inhibitors may also help reduce tumor-induced systemic inflammation and improve blood flow in peripheral arteries.[43] The PAD4 gene is linked to epigenetic and phenotypic changes in several cancers.[44-46] Increased PAD4 expression enhances the malignant properties of NPC cells. GSK484 reduces citH3 protein levels, a marker of PAD4 activity, and inhibits NPC progression by suppressing PAD4's citrullination.[47] Since GSK484 is a reversible inhibitor, extended exposure may not entirely inactivate PAD4. Nevertheless, GSK484 notably inhibited tumor growth in vivo, suggesting PAD4 as a potential biomarker for NPC treatment. F-amidine, with its ability to irreversibly inhibit PAD4, may offer benefits over GSK484.[47] Furthermore, PAD4 is highly expressed in stomach cancer, driving cell proliferation, and its inhibitors could help prevent cancer metastasis.[46]

Inflammation is a key factor in placentation problems, including miscarriages, fetal growth restriction, and preeclampsia.[48] In pregnant mice, overproduction of soluble Flt-1 (sFlt-1) leads to issues ranging from spontaneous fetal loss to preeclampsia, with the severity dependent on the dosage.[49] Alterations in sFlt-1 and other angiogenic proteins, such as placental growth factor, have been associated with various reproductive conditions, including miscarriages, fetal growth limitation, and preeclampsia.[50-52] Sarcoma-like tyrosine kinase 1 (sFlt-1) induces significant neutrophil accumulation and NET formation in mouse placentas. In a mouse model with sFlt-1 overexpression, the absence of PAD4 reduces both placental and systemic inflammation, protecting against pregnancy loss caused by sFlt-1.[53] The pregnancy loss linked to sFlt-1 is associated with PAD4-triggered NETosis. Thus, inhibiting PAD4 may offer a novel strategy to prevent inflammatory miscarriages during early placentation defects, potentially preserving pregnancies.

Retinal stress and disease induce excessive production of glial fibrillary acidic protein (GFAP), which undergoes citrullination in the damaged retina.[54,55] Inhibiting PAD4-mediated citrullination results in a significant decrease in GFAP expression, indicating that targeting GFAP could be a promising treatment strategy for retinopathy.[56] PAD4 is crucial in inflammation during ischemic acute kidney injury (AKI), promoting renal tubular inflammation, neutrophil infiltration, and NFκB activation.[57] In mice, PAD4 activity increases following renal ischemia-reperfusion (I/R) injury. After 30 minutes of ischemia, untreated mice exhibited severe AKI with elevated plasma creatinine levels. Pretreatment of mice with PAD4 inhibitors (2-chloroamidine or streptonigrin) led to a significant reduction in I/R injury.[58] These findings emphasize PAD4's role in enhancing inflammatory responses and neutrophil infiltration during renal I/R injury.[58] Liver I/R injury initiates the release of damage-associated molecular patterns, triggering an immune response. Treatment with PAD4 inhibitors or DNase I significantly protects hepatocytes and reduces inflammation by inhibiting NET formation, underscoring their role in liver I/R injury. PAD4 inhibitors and DNase I reduce liver damage mediated by High Mobility Group Box 1 and histones.[59] The PAD4 inhibitor YW4-03 notably decreased liver I/R injury, suggesting its potential as a therapeutic agent. Targeting NETs with PAD4 inhibitors or DNase I may improve outcomes in liver surgery patients at risk of ischemia/reperfusion injury.[59]

In systemic lupus erythematosus (SLE), patients have an impaired ability to break down NETs, which can result in increased exposure of nuclear antigens to the immune system. Suppressing PAD activity significantly alleviates vascular, renal, and skin disease in lupus-prone mice by reducing NET formation.[60,61] However, PAD4 deletion in glomerulonephritis models did not improve nephritis or immune activation, and PAD inhibition had no effect on end-organ damage. Treatment of mice in New Zealand with Cl-amidine reduced NET formation, altered autoantibody profiles, decreased complement levels, and lowered IgG deposition in the glomeruli, suggesting potential therapeutic benefits in SLE.[62,63]

Cl-amidine not only reduced NET formation but also enhanced bone marrow endothelial progenitor cell differentiation,

improved endothelial-dependent blood vessel relaxation, and delayed arterial thrombosis after photochemical exposure.[63] In SLE, dysfunctional NET degradation leads to an accumulation of NETs, which can trigger Type I IFN responses, activate inflammasomes, and cause endothelial damage, contributing to abnormal immune responses.[64-66] These findings suggest that inhibiting PAD activity may help modulate the development and severity of lupus and its symptoms.

Elevated amounts of ACPAs have been seen in the bloodstream of a xenografted animal model of ulcerative colitis.[67] Therefore, it is hypothesized that the development of ulcerative colitis may be attributed to an excessive level of citrullination. In Alzheimer's disease (AD), PADs and citrullinated proteins are more prevalent in brain regions affected by neurodegeneration. Neuronal death releases citrullinated proteins into the interstitial space, which can enter the bloodstream and lymphatic system, triggering an immune response. This results in persistent exposure of the central nervous system to the immune system.[68]

PAD4, citrullinated peptides, and NETs have been found in atherosclerotic plaques, suggesting that immune complexes at these sites may drive inflammation and disease progression.[69,70] A study of 134 female RA patients showed a strong link between ACPA levels and subclinical atherosclerosis. Recent research further confirmed NETs' role in atherosclerosis, showing that cholesterol crystals trigger NETosis, IL-1b production, and TH17 activation, which recruit immune cells.[70] In mouse models, PAD inhibition reduced NET formation, lesion size, and thrombosis risk while also decreasing immune cell recruitment and interferon-α production in arteries.[71] Inhibiting PAD4 could thus reduce NET formation and immune activation, offering a potential therapeutic approach.

Protein citrullination was once thought to be a bacterial strategy to evade immune detection by deactivating NETs.[72] However, experiments showed that inhibiting PAD4 had no effect on amoebic NETosis, while calcium chelation inhibited the process, as calcium is crucial for neutrophil adhesion during NETosis.[73] The PAD4 inhibitor GSK484 also failed to suppress NET release triggered by amoebas, suggesting that while citrullination plays a role in NETosis, it may not be involved in amoebic NETosis, which likely occurs via a different mechanism.[74] In contrast to Staphylococcus, PAD4 inhibition reduced phagocytosis and oxidase activation.[75] PAD2 levels are markedly higher in the LβT2 gonadotrope cell line compared to other PAD isoforms, with peak expression occurring during estrus. This indicates that PADs may be involved in regulating gonadotropin synthesis in females, potentially offering a new approach to controlling fertility through PAD-catalyzed citrullination in gonadotropes.The functional overlap between PAD2 and PAD4 may explain why PAD2 or PAD4 knockout mice have limited utility in reproductive studies, as they seem to compensate for each other's roles.[76,77]

NETs facilitate thrombosis by serving as a scaffold for fibrin deposition and enhancing platelet adhesion, aggregation, and activation. Mice deficient in PAD4 are protected from DVT in a stenosis model, with no extracellular citrullinated histones detected despite neutrophil mobilization. Infusion of wild-type neutrophils restored thrombosis, highlighting the crucial role of PAD4 in neutrophils for thrombosis. Advanced imaging has replicated DVT dynamics in mice, showing that high interstitial hemodynamic forces trigger NET release in sterile occlusive thrombosis, though PAD4 inhibition did not prevent NETosis.[78-80] PAD4 in the bloodstream impairs the clearance of VWF-platelet strings and accelerates platelet plug formation after vessel damage, partly by reducing ADAMTS13 activity.[81,82] The enzyme r-huPAD4 converts specific arginine residues on ADAMTS13 into citrulline, significantly decreasing its activity. Plasma samples from patients with sepsis or elderly individuals with comorbidities (e.g., diabetes, hypertension) show increased citrullination of ADAMTS13 compared to healthy controls.[83]

NET levels are elevated in nonhealing diabetic foot ulcers. In mice, diabetes increases skin PAD4 activity, leading to histone citrullination. Inhibiting PAD4 with Cl-amidine reduced neutrophil-derived NETs and improved wound healing. Cl-amidine may, therefore, be a promising topical treatment for diabetic individuals.[84] YW3-56, a PAD2/PAD4 inhibitor, reduces lipopolysaccharide (LPS)-induced pulmonary dysfunction by decreasing NET formation and CitH3 generation. This compound helps preserve endothelial cell integrity, reduces pulmonary vascular dysfunction, and alleviates acute lung injury (ALI), improving survival in a mouse model of endotoxemia.[85] In another experiment, TDFA, a PAD4-specific inhibitor, reduced lung edema, pulmonary damage, and mortality after LPS exposure. TDFA also suppressed inflammation by lowering pro-inflammatory cytokines and oxidative stress, suggesting a potential novel treatment for LPS-induced ALI.[86]

Mice lacking PAD4 and infected with bacteremia showed similar survival rates to normal mice, with no worse outcomes. Antibiotic treatment in PAD4-deficient mice slightly increased the death rate, but bacterial presence in the bloodstream remained unchanged, and there was partial protection against LPS-induced shock.[87] These findings suggest that PAD4/NETs-mediated toxic inflammation is driven by bacterial endotoxins, and inhibiting PAD4 to prevent NET formation in inflammatory disorders is unlikely to increase infection susceptibility.[87] A study of 127 patients found that high PAD4 levels, but not specific PAD4 polymorphisms (PAD4_89, PAD4_94, PAD4_104), were linked to increased mortality in septic shock patients in the ICU.[88]

PAD4 influences key transcription factors like NFkB and interacts with the apoptotic regulator p53, playing a role in inflammatory gene production.[89] It also regulates CXCL chemokine production in human macrophages, especially

when stimulated by TLR ligands or *Plasmodium falciparum*. CXCL1 may serve as a biomarker for severe malaria. Beyond neutrophil regulation, PAD4 controls chemokine synthesis in macrophages, with its inhibition delaying liver cell movement. While blocking PAD4 doesn't significantly affect individual chemokines, its overall impact on multiple chemokines may explain this delay. PAD4's role in macrophage death could further explain the observed delays in CXCL1 and CXCL2 production when PAD4 is inhibited.[90,91]

Patients with acute pulmonary embolism (APE) display elevated levels of NETs and lysophosphatidic acid (LPA). Autotaxin, the enzyme responsible for producing LPA, is present in intrapulmonary thrombi and surrounds NETs. LPA rapidly induces NET release through a PAD4-dependent pathway, and these LPA-induced NETs contribute to a thrombus that is resistant to tissue plasminogen activator (tPA). This suggests that PAD4 inhibitors may be necessary to overcome tPA resistance in APE.[92] PAD4 is crucial for NET formation and the release of DNA in neutrophils. In individuals with cystic fibrosis (CF), higher levels of anti-PAD4 antibodies are linked to lung infections caused by Pseudomonas aeruginosa. Neutrophils are the primary source of PAD4 in CF, as they are abundant in the airways, express high levels of PAD4, and play a key role in NET release.[93,94]

PAD2 and PAD4 enzymes are present in the salivary glands of Balb/c mice, but their ability to citrullinate Ro and La ribonucleoproteins remains uncertain. In Sjögren's syndrome, PTMs in the salivary glands may rely on PAD activity. These modifications suggest that external triggers, such as bacteria or chemical compounds, can activate the PAD pathway, transforming normal proteins into potential autoantigens. This process may stimulate autoimmune class II MHC molecules and autoreactive cell clones, initiating an autoimmune response akin to Sjögren's syndrome.[95]

P. gingivalis, linked to severe periodontitis, secretes an enzyme that converts peptidyl arginine into citrulline residues. To survive the influx of neutrophils in inflamed gum tissue, *P. gingivalis* uses Porphyromonas Peptidylarginine Deaminase (PPAD) to prevent neutrophil death. PPAD also modifies proteins, including certain integrins, to facilitate their uptake by phagocytes. If bacterial PPAD shares homology with human PADs, inhibitors of human PADs could be repurposed as antibiotics. However, if *P. gingivalis* has a distinct active site, adapting existing PAD inhibitors could offer a novel approach to targeting this pathogen.[96]

THERAPEUTIC TARGETING OF PAD4 IN RA

Cells of myeloid lineage, including neutrophils, eosinophils, and monocytes/macrophages, express PAD4, which is believed to regulate gene expression, cell division, programmed cell death, NETs, and tumor development. PAD4 plays a crucial role in cardiovascular diseases, autoimmune disorders, and cancers, making it a promising target for diagnosis and treatment.[97] Small-molecule inhibitors of PAD, such as BB-Cl-amidine and the PAD4-specific GSK199, show therapeutic potential and aid in citrullination research. Using an ELISA assay, Martín Monreal *et al*. discovered that AFM-30a effectively inhibited PAD activity in intact PMNs, lysed PBMCs, and synovial fluid from RA patients, while GSK199 had minimal effect.[98] In addition, AFM-30a effectively inhibits PAD2 in PBMCs, PMNs, and synovial fluid, as demonstrated by Martín Monreal *et al*. When combined with GSK199, AFM-30a reduces PAD activity in PBMCs without the cytotoxicity observed with BB-Cl-amidine. These findings suggest that AFM-30a and GSK199 may have fewer side effects, making them potentially more suitable for therapeutic use.[98]

A preliminary study indicates that anti-PAD4 monoclonal antibodies may enhance the production of pro-inflammatory cytokines in monocytes, potentially transforming them into bone-resorbing cells, a process associated with RA.[99] The precise mechanism involving autoantibodies remains unclear. However, the presence of PAD4 on monocyte surfaces and the binding of ACPAs to immature DC-derived osteoclasts suggest that these antibodies could initiate pathogenic effects.[99,100] This interaction may activate NK cells, inducing antibody-dependent cytotoxicity through perforin, granzyme, or complement activation, leading to sustained histone release and the production of citrullinated RA autoantigens by neutrophils.[101]

Comprehending immune responses to citrullinated proteins and PAD enzymes could pave the way for novel treatments that decouple inflammatory and degenerative effects from PAD enzyme function. Researchers have suggested using chimeric autoantibody receptor T-cell vaccines to target specific antibodies in autoimmune disorders.[102] In a safety trial, Benham *et al*. discovered that Rheumavax, a personalized dendritic cell vaccine aimed at inducing tolerance to citrullinated peptides, exhibited immunomodulatory and anti-inflammatory effects in RA patients.[103] With a deeper understanding of the specific immunogenic epitopes involved in RA, targeted immunotherapies could be developed to suppress autoreactive T and B cells while preserving PAD enzyme function.[5] Antibodies targeting PAD4 and PAD2 have been identified in certain groups of RA patients, and these autoantibodies could serve as predictive biomarkers. Incorporating anti-PAD antibodies alongside ACPAs, RF, and other autoantibodies could aid in better categorizing RA patients. However, further research is needed to fully understand the regulation of PAD4 activity.[5] Liu *et al*. emphasized the importance of developing PAD4 inhibitors, as PAD4 is implicated in multiple disorders. Despite advances in creating PAD4 inhibitors targeting specific isotypes, none are currently available for clinical use.[104] Further research into PAD4's structure, function, and regulation is crucial for designing effective inhibitors to treat autoimmune and inflammatory diseases.

POTENTIAL SIDE EFFECTS AND SAFETY CONCERNS

PAD4 inhibitors offer potential for treating various conditions, but like any medication, they may have side effects. Still in the experimental phase, their safety and effectiveness have not been fully established.[36] Research has shown that mice without PAD are more prone to bacterial infections, indicating that inhibiting PAD activity and NET formation could increase infection risk.[36] Thanabalasuriar *et al*. found that PAD4-deficient mice exhibited extensive inflammation and bacterial keratitis, an eye infection.[105]

Lu *et al*. used the PAD4 inhibitor YW3-56 with an Au nanodrug delivery system to target tumors and induce cell apoptosis through chemical-photothermal therapy, significantly inhibiting tumor growth and lung metastasis in mice with good biosafety.[106] Gajendran *et al*. also found that the PAD4 inhibitor JBI-589 did not negatively impact body or spleen weight in mice.[107] However, no PAD4 inhibitor has been approved for human use, emphasizing the need for further development of inhibitors with optimal ADME properties, safety, and efficacy.[107] Yu *et al*. created both reversible (e.g., GSK199 and GSK484) and irreversible (e.g., F- and Cl-amidine) PAD4 inhibitors effective against inflammation and cancer, which are in preclinical stages.[80] Future research should aim to discover safer and more effective drugs for clinical use, with a thorough understanding of PAD4 to avoid side effects like increased infection risk. Yang *et al*. showed that GSK199 was effective in preventing murine arthritis.[7,8] To minimize infection risks, future PAD4 inhibitors should be designed to act extracellularly and avoid entering cells.

CONCLUSION

In conclusion, this review highlights the significance of PAD4 as a promising therapeutic target for RA and other related conditions. PAD4's role in citrullination and the formation of autoantibodies underscores its potential for targeted intervention in RA and associated diseases. While targeting PAD4 offers significant therapeutic promise, future research and clinical trials must address challenges related to specificity, safety, and long-term efficacy. A comprehensive understanding of PAD4's diverse functions, beyond its enzymatic activity, is essential to fully explore its therapeutic potential.

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