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# Pathological and therapeutic aspects of matrix metalloproteinases: implications in childhood leukemia

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## Abstract

Matrix metalloproteinases (MMPs) play a major role in extracellular matrix remodeling and are involved in tumor cell invasion. Cancers such as childhood leukemia are characterized by their capacity to infiltrate different organs. MMP production by leukemic cells may indicate a leukemic subtype or subpopulation with a more invasive phenotype. Therefore, clarifying the action mechanisms of MMPs as prognostic predictors or MMP targeting as a therapeutic strategy is necessary. MMP-targeting drugs have been developed for the treatment of hematological malignancies. In this review, we highlight current advances in understanding the molecular mechanisms and pathological characteristics of various MMPs, as well as recent therapeutic advances targeting MMPs in childhood leukemia. Several studies have been conducted on the therapeutic efficacy of MMP inhibitors in cancer, such as collagen peptidomimetics, nonpeptidomimetic inhibitors of MMP active sites, bisphosphonates, and tetracycline derivatives. Here, we conclude that more clinical trials are necessary to estimate the role of selective MMP inhibitors in the treatment and prevention of childhood leukemia.

**Keywords** Matrix metalloproteinases · Cancer progression · Metastasis · Childhood leukemia

## 1 Introduction

Cancer is the second most common cause of death in children in developed countries [1]. Leukemia accounts for 25–35% of all cases of childhood cancer in many populations and is thus the most common type of cancer in children [1, 2]. Acute myeloid leukemia (AML) and acute lymphocytic leukemia (ALL) are two major subtypes of childhood leukemia and account for approximately 76% of all pediatric leukemias [3, 4]. Extensive research has characterized the risk factors for childhood leukemia, such as exposure to carcinogens or chemicals, as well as potentially chemopreventitive activities [5]. However, the exact etiology of childhood leukemia remains poorly understood as the molecular mechanism underlying disease progression has not been adequately studied. One study reported that a combination of treatment protocols and risk-adapted treatment intensity led to a long-term survival rate of 80–90% in patients with ALL and 60–70% in patients with AML [6]; however, this study could not precisely identify children at risk of treatment failure on the bases of prognostic factors, including patient-related and leukemia-related factors, and morphological assessment of each individual treatment response. Relapse occurrence also poses a challenge to the development and implementation of treatment strategies [7]. Therefore, identifying novel biomarkers and therapeutic approaches for the diagnosis, prognosis, and management of childhood leukemia is crucial. Understanding the biology and targeting molecular

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pathways may facilitate the development of novel therapies for clinical application or intervention to prevent relapse [8–12].

Matrix metalloproteinases (MMPs), a family of zinc-dependent endopeptides, play a major role in extracellular matrix (ECM) remodeling and are involved in tumor cell invasion [13–18]. Among MMPs, gelatinase (MMP-2 and MMP-9) production by leukemic cells can help identify a leukemic subtype or subpopulation with a more invasive phenotype [19]. MMP-2 and MMP-9 share a unique catalytic domain among MMPs: they both harbor the fibronectin repeat domain, which can bind and degrade type IV collagen and denatured gelatin. MMP-9 additionally has a unique type V collagen-like domain (Fig. 1). A prospective study on childhood ALL revealed that a high secretion of MMP-9 was associated with a lower overall survival rate [20]. MMP-9 could be an independent prognostic factor in childhood B cell ALL (B-ALL) [20]. MMP genetic variants have been reported to contribute to the risk of childhood leukemia [21–24]. In addition to childhood leukemia, several studies also demonstrated that MMPs also play a role in adult leukemia [25–27]. Gusella et al. showed plasma MMP-9 levels positively correlated with blood lymphocytosis, particularly in more advanced stages in the 36 adult patients with B cell chronic lymphocytic leukemia [25]. Moreover, a study surveyed MMP-2 and MMP-9 expressions in 20 adult and 55 pediatric acute lymphatic leukemia (ALL) patients. In adult ALL, there was a significant correlation between MMP-2 positivity and extramedullary infiltration [27]. Therefore, clarifying the role for MMPs as a prognostic predictor or as a target for anti-cancer therapy is necessary. In this review, we discuss the mechanisms of MMPs in the progression of childhood leukemia and the use of therapeutic agents that target MMPs in the treatment of these diseases.

## 2 Mechanisms of MMPs in the progression of childhood leukemia

Several studies have demonstrated that MMPs are involved in leukemic cell migration, invasion, and metastasis [28–31]. Table 1 and Fig. 2 summarize the mechanisms through which MMPs are regulated in the progression of childhood leukemia. Among MMPs, MMP-9 is a zinc-dependent endopeptide for collagen and gelatin and mainly participates in cell migration, invasion, and metastasis. Zhu et al. demonstrated the recruitment of hematopoietic stem cells from bone marrow (BM) in chronic myeloid leukemia (CML) [28]. They determined that the breakpoint cluster region/Abelson murine leukemia (*BCR/ABL*) oncogene induces MMP-9 production through the phosphatidylinositol-3 kinase (PI3K)/protein kinase B/nuclear factor (NF)-κB signaling pathway. Moreover, MMP-9, induced by transforming growth factor-beta1, may upregulate soluble-intracellular adhesion molecule-1 expression, which plays a critical role in this signaling pathway and in the recruitment of malignant cells in CML. Zhu et al. moreover concluded that MMP-9 could facilitate hematopoietic stem cell transition from quiescence to the proliferative niche in CML [28]. MMP-9 in B cell chronic lymphocytic leukemia (B-CLL) is also involved in cell invasion and migration [29]. Redondo-Munoz et al. established that through the alpha4beta1 integrin and C-X-C motif chemokine 12 (CXCL12) axis, MMP-9 plays a key role in cell migration, thus contributing to B-CLL progression [29].

A study demonstrated that MMP-9 could promote cell migration through noncatalytic mechanisms by activating rho-associated protein kinase pathways after binding CD44 on cancer cells [32]. Redondo-Munoz et al. identified a noncatalytic role of MMP-9 in another process critical to cancer progression and cell proliferation [30]. They

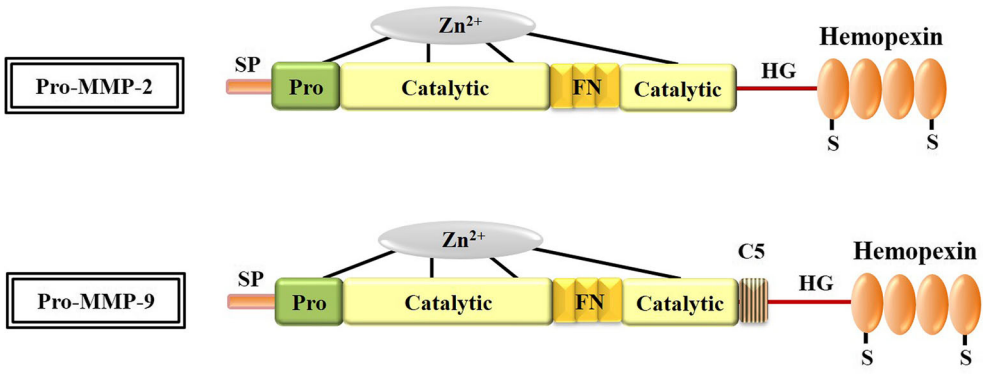


**Fig. 1** Schematic structure of matrix metalloproteinases MMP-2 and MMP-9

**SP: Signal sequence**
**Pro: Pro-peptide with zinc-ligating groups**
**FN: Fibronectin repeats**
**C5: Type-V collagen-like domain**
**HG: Hinge region**

**Table 1** Regulation of MMPs in childhood leukemia

| Cell type | MMP | Pathway | Target molecules | References |
|---|---|---|---|---|
| Chronic myeloid leukemia | MMP-9 | PI3K/Akt/NF-κB signaling pathway | s-ICAM-1 | Zhu B et al. 2015 [28] |
| B cell chronic lymphocytic leukemia | MMP-9 | PI3K/Akt/NF-κB signaling pathway | CXCL12 | Redondo-Munoz J et al. 2006 [29] |
| Chronic lymphocytic leukemia | MMP-9 | Stat3 signaling pathway | α4β1 integrin/CD44v | Redondo-Munoz J et al. 2010 [30] |
| Acute monocytic leukemia cell | MMP-2/MMP-9 | Tight junction proteins pathway | ZO-1, claudin-5 | Feng S et al. 2011 [31] |

*MMP-2* matrix metalloproteinase-2, *MMP-9* matrix metalloproteinase-9, *PI3K* phosphatidylinositol-3 kinase, *NF-κB* nuclear factor (NF)-κB, *s-ICAM-1* soluble-intracellular adhesion molecule-1, *CXCL12* C-X-C motif chemokine 12

demonstrated that in CLL, α4β1 integrin and CD44 bind to the hemopexin domain of pro-MMP-9 and subsequently activate antiapoptotic pathways through Stat3 phosphorylation [30]. These results indicate an important role for MMP receptor binding and induced intracellular survival signals in B-CLL pathogenesis [30].

Central nervous system (CNS) involvement is a major cause of mortality and morbidity in childhood leukemia. The

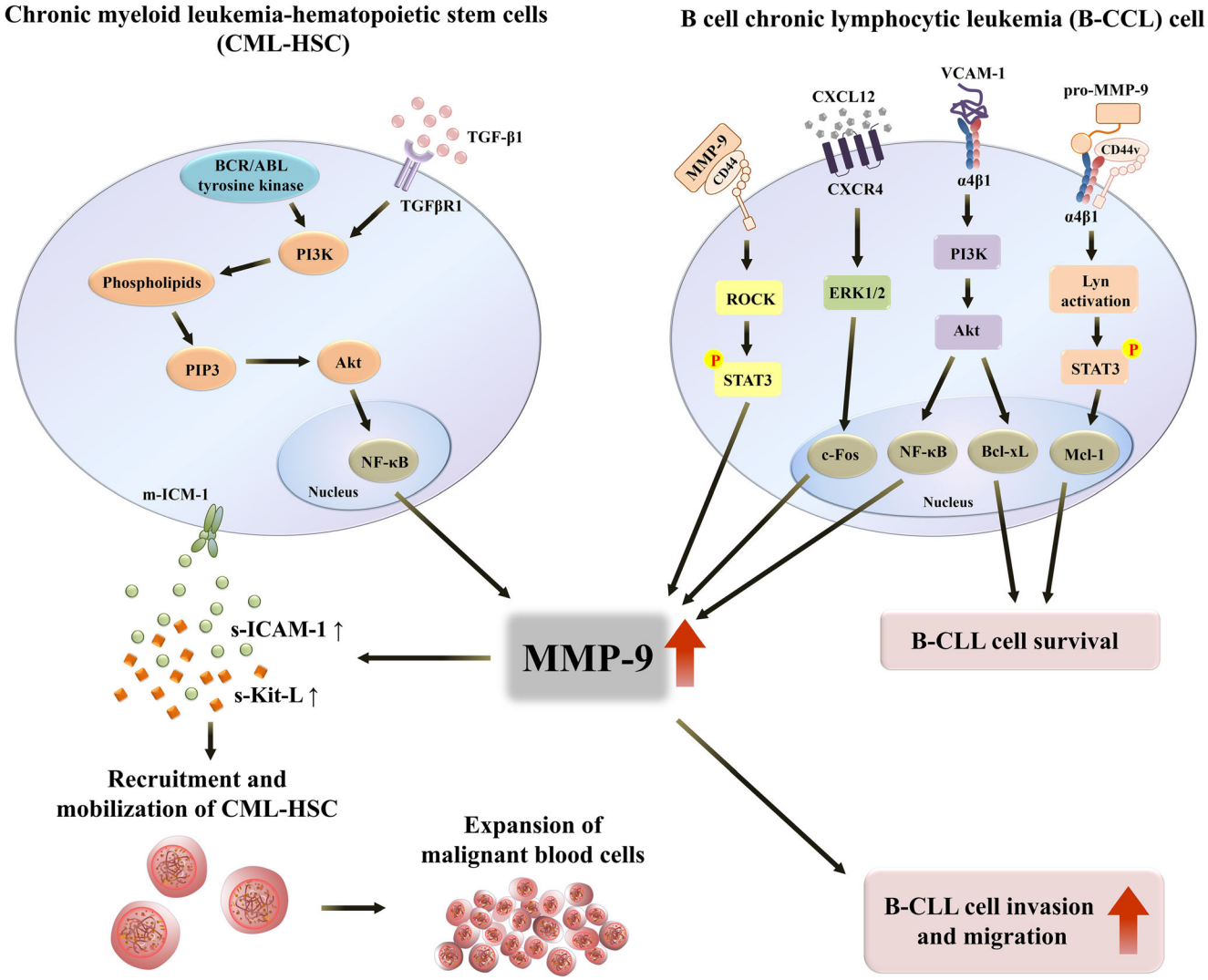


**Fig. 2** A summary of various signaling pathways involved in MMP-9 on human chronic myeloid leukemia. MMP-9, matrix metalloproteinase-9; phosphatidylinositol-3 kinase, PI3K; NF-κB, nuclear factor (NF)-κB; s-ICAM-1, soluble-intracellular adhesion molecule-1; CXCL12, C-X-C motif chemokine 12

mechanisms through which leukemic cells disrupt the blood–brain barrier (BBB) and infiltrate brain and surrounding tissues are outlined as follows: leukemic cells impair the tight junction proteins zonula occludens-1, claudin-5, and occludin, resulting in increased permeability of the BBB. Furthermore, MMP-2 and MMP-9 mediate the BBB opening by disrupting tight junction proteins in the CNS in leukemia [31]. Accordingly, MMP-2 and MMP-9 secreted by leukemic cells may increase BBB permeability by disrupting tight junction proteins [31].

## 3 Pathological aspects and clinical relevance of MMPs in childhood leukemia

Several studies have demonstrated the clinical relevance of MMPs in leukemia [33–35]. Importantly, adult and childhood ALL differ in terms of their biological characteristics [27]. Kuittinen et al. studied MMP-2 and MMP-9 expressions in 20 adult and 55 pediatric patients with ALL and observed that MMP-2 positivity significantly correlated with the appearance of extramedullary infiltrates in adult ALL, suggesting MMP-2 and MMP-9 activities might facilitate increased extravasation of leukemic cells in ALL [27]. However, they reported that only 7 (12.7%) of the 55 patients had positive immunostaining for MMP-2 or MMP-9 expressions. Accordingly, the biological characteristics of adult and childhood ALL are different.

Other studies have identified a link between MMPs and the progression of pediatric leukemias. Song et al. found that increased cell migration and drug resistance lead to poor prognosis in acute myeloid leukemia cells [33]. They moreover discovered increased MMP-2 expression and gelatinolytic activity, and subsequent increased tumor invasiveness, in drug-resistant variants of the human acute myeloid leukemia cell line AML-2/WT. Poor prognosis was due to increased cell migration engendered by ECM degradation through increased MMP-2 expression as well as drug resistance [33]. An increase in MMP-2 expression may promote leukemic cell extravasation and thus could be associated with hepatosplenomegaly [34]. Abnormal MMP-2 and MMP-9 expression levels were observed in patients with AML and in those with myelodysplastic syndrome (MDS) [35]. Thus, MMP production and release may influence hematopoietic cell behavior [35]. This study and others suggest that MMP expression could be both a diagnostic and prognostic tool in the future [35].

Leukemic cells proliferate in BM and are released into the peripheral blood [36]. Within the context of cancer, the proteolytic activities of MMPs function to remove physical barriers and facilitate tumor cell migration and invasion of surrounding tissues [37]. Primary human AML cells may release several MMPs and chemokines constitutively, as extensive crosstalk exists between the MMP system and chemokine network [37]. The NF-κB system represents a regulator for MMPs (e.g., MMP-1 and MMP-9) and chemokines (CCL2–4/CXCL1/8) through primary human AML cells [37]. Because the chemokine network and MMPs interact at the functional level, these two systems involve AML cell proliferation and migration [37].

One important study investigated the involvement of circulating MMP-2 in the prognosis of patients with AML [38]. In the study, patients with AML were divided into two groups according to the median pretreatment level of MMP-2: high and low MMP-2 groups. The survival period of patients in the high MMP-2 group was shorter than that of those in the low MMP-2 group [38]. Studies have shown that the expression of MMPs, especially MMP-2 and MMP-9, is associated with leukemia progression [36, 39, 40]. In myeloid cells, MMP-2 has a low expression level; MMP-9 is mainly expressed in T lymphocytes, malignant B lymphocytes, and mature myeloid and monocytic cells [36, 39]. Scrideli et al. examined the mRNA expression of MMP-2, MMP-9, tissue inhibitor of metalloproteinase-1 (TIMP-1), and TIMP-2 in 134 children with ALL [41]. They revealed that higher TIMP-1 expression levels in ALL blast cells at diagnosis were associated with unfavorable clinical courses in children with ALL [41]. A complex interaction was observed between migration/adhesion genes and childhood ALL [41].

Ries et al. demonstrated that BM mononuclear cells continuously produced MMP-9 and its inhibitor TIMP-1, and that leukemic blast cells expressed and secreted MMP-2 [42]. They argued that MMP-2 could be a marker of malignant transformation in AML and a prognostic factor for disease progression in CML and MDS. The release of MMP-9 and MMP-2 from leukemic blasts could cause leukemic cell dissemination through the local degradation of ECM barriers followed by the invasion and metastasis of tumor cells [42].

The clinical implications of MMP expression in leukemia may depend on patients' physiological status [36]. Genetic variation of MMPs may affect the incidence of childhood ALL [36]. Lin et al. determined that MMP-2–1306 C/T and MMP-9–1562 C/T single-nucleotide polymorphisms may be associated with a higher incidence of T-ALL; MMP-9–1562 C > T polymorphism may be related to the prognosis of patients with T-ALL [24]. In patients with childhood ALL, rs3216144 and rs10502001 polymorphisms of MMP-7 were associated with the risk of relapse [43].

Another study found that MMP-9 levels in the BM of patients with AML and ALL were lower than those in controls, and that patients who exhibited a complete response to therapy had the same range of MMP-9 expression as controls [44]. Therefore, MMP-9 could be a biomarker to monitor the status of patients with AML [44]. MMP-9 expression was also reported to be high in AML patients with extramedullary infiltration than in those without such infiltration [36, 45]. The infiltration may be related to the release of LCs from BM to the peripheral blood [45].

An increase in MMP-2 expression in patients with AML was correlated with AML blast cell invasion, leukemogenesis, and possibly chemosensitivity [46]. Furthermore, drug-resistant cell lines were reported to exhibit predominant increases in MMP-2 expression, MMP-2 gelatinolytic activity, and tumor invasiveness [33]. Aref et al. demonstrated the prognostic relevance of MMP-2 in the clinical outcomes of patients with AML [38]. High pretreatment levels of MMP-2 were associated with poor survival in patients with AML [38].

Chen et al. revealed that NF-κB- and activator protein (AP)-1-mediated DNA looping regulates MMP-9 transcription in TNF-α-treated human leukemia U937 cells [47]. They also revealed that TNF-α-stimulated MMP-9 promoter activity was regulated through NF-κB/p65 and AP-1/c-Jun [47]. Membrane type-1 MMP (MT1-MMP) has been implicated in tumor invasion and acts as a physiologic activator of pro-MMP-2. Marquez-Curtis et al. examined MT1-MMP expression in primary AML cells and the effect of TNF-α on MT1-MMP expression [48]. Recombinant TNF-α-upregulated MT1-MMP expression in AML cells leads to the enhancement of pro-MMP-2 activation and trans-Matrigel migration [48]. AML cells express MT1-MMP and TNF-α, resulting in increased MMP-2 activation and contributing to an invasive phenotype [48]. An increase in TNF-α can change the expression of MMPs, thus affecting LC invasion [36]. Moreover, Sato et al. identified tetraspanins as binding proteins of MT1-MMP that enable efficient MMP-2 activation and proteolysis coupled with cellular function [49].

Recent research has revealed the potential roles of MMP-7 and MMP-15 in the prognosis of AML [50, 51]. MMP-7 and MMP-15 were overexpressed in patients with AML; high MMP-7 or MMP-15 expression in the BM of patients with AML predicted poor overall survival [50]. Furthermore, multiple long noncoding RNAs (lncRNAs) were correlated with MMP-7 and MMP-15; lncRNAs could be involved in the pathogenesis of AML through MMP modulation [50]. Wu et al. reported that MMP-7 and phosphatase and tension homolog levels in serum samples of control and complete remission groups were significantly increased relative to those in an incomplete remission group [51].

## 4 Therapeutic agents targeting MMPs in the treatment of childhood leukemia

MMPs have been considered as attractive cancer targets [52]. Some drugs, such as imatinib mesylate, may affect the expression of MMPs [53]. The imbalance between the expression of MMPs and their inhibitors may promote leukemia cells migration, angiogenesis, survival, or apoptosis [36]. MMP inhibitors, broad-spectrum drugs designed as antitumor agents, have been validated [52]. Several types of agents targeting MMPs have been investigated and validated for their potential in childhood leukemia treatment. Table 2 presents MMP-targeting therapeutic agents used in the treatment of childhood leukemia.

The mechanisms through which leukemic cells infiltrate the CNS are outlined as follows: leukemic cells secrete MMP-2 and MMP-9, and this increases the permeability of the BBB by disrupting tight junction proteins [31]. MMP-2 and MMP-9 were revealed to be upregulated in mouse brain tissues with leukemic cell infiltration [31]. However, these activities were reported to be reduced when MMP-2 and MMP-9 were inhibited by the MMP inhibitor GM6001 in an *in vitro* BBB model [31]. GM6001 is one of the first MMP inhibitors to go into clinical trials. Both safety and efficacy were validated in these phase I and phase II trials [59]. Accordingly, GM6001 was determined to have protective effects against CNS leukemia [31].

Cpd 11, a pentanoic acid derivative targeting MMP-2, induces apoptosis in a CML cell line [54]. Cpd 11 could downregulate MMP-2 expression, thus demonstrating anti-invasive and apoptotic activity in a K562 cell line [54]. Therefore, Cpd 11 is a potent MMP-2 inhibitor that can block the invasiveness of K562 cells [54].

Extramedullary infiltration (EMI) is associated with the prognosis of leukemia. Patients carrying the acute myeloid leukemia 1 protein/protein ETO (AML1/ETO; A/E) fusion gene and expressing the amyloid precursor protein (APP) tended to develop EMI and had a poor prognosis [60]. Jiang et al. designed microRNA-144 (miR-144) mimics and transfected leukemia Kasumi-1 cells. They demonstrated that microRNA-144 (miR-144) negatively targets APP and regulates AML1/ETO(+) leukemia cell migration through the APP/p-ERK/c-Myc/MMP-2 pathway [60].

A study investigated the effect of the adenovirus-mediated TIMP-3 on the *in vitro* growth and invasiveness of chronic myelogenous leukemia K562 cells and on the *in vivo* progress of K562-derived xenografts in nude mice [61]. The study noted no direct effect of TIMP-3 overexpression on the growth of K562 cells *in vitro*; however, repeated intratumoral injection of Ad-TIMP-3 inhibited the growth, lowered the microvessel density, reduced the vessel maturity, and increased the apoptosis of K562 xenografts in nude mice [61].

A recent study reported that doxycyclineattenuated leukemic cell migration in the leukemic cell lines KG1a (acute myelogenous leukemia) and K562 (chronic myelogenous leukemia) [55]. The effect was associated with the inhibition of the MMP-2 and MMP-9 expression and phosphorylation of focal adhesion kinase. Accordingly, doxycycline has potential for leukemia treatment.

Another study reported amsacrine to attenuate cell invasion, inhibit MMP-2 and MMP-9 expressions, and regulate mRNA levels in human leukemia cells [56]. Amsacrine-induced MMP-2 and MMP-9 downregulation was revealed to be related to protein phosphatase 2A catalytic subunit α upregulation in human leukemia cells [56]. Amsacrine

**Table 2** Therapeutic agents targeting MMPs in childhood leukemia

| Therapeutic agents | Experimental leukemic cell line(s) | Targeted MMP(s) | Mechanism of action | References |
|---|---|---|---|---|
| GM6001 | Acute monocytic leukemia cell (SHI-1 cell line) | MMP-2/MMP-9 | Tight junction proteins pathway | Feng S et al. 2011 [31] |
| Cpd 11 | Chronic myeloid leukemia cell (K562 cell line) | MMP-2 | Apoptosis pathway | Mukherjee A et al. 2017 [54] |
| Doxycycline | Acute myelogenous leukemia cell (KG1a cell line)/chronic myeloid leukemia cell (K562 cell line) | MMP-2/MMP-9 | FAK signaling pathway | Wang C et al. 2015 [55] |
| Amsacrine | Human leukemia cell (U937, Jurkat, HL-60, K562, KU812, and MEG-01 cells line) | MMP-2/MMP-9 | p38 MAPK/JNK signaling pathway | Liu WH et al. 2014 [56] |
| Caffeine | Human leukemia cell (U937 cells line) | MMP-2/MMP-9 | p38 MAPK/c-Jun signaling pathway | Liu WH et al. 2010 [57] |
| Gallic acid | Chronic myeloid leukemia cell (K562 cell line) | MMP-2/MMP-9 | Akt/ERK/c-Fos signaling pathway | Chen YJ et al. 2012 [58] |

*MMP-2* matrix metalloproteinase-2, *MMP-9* matrix metalloproteinase-9, *PI3K* phosphatidylinositol-3 kinase, *FAK* focal adhesion kinase, *ERK* extracellular signal-regulated kinases

suppresses MMP-2/MMP-9 promoter luciferase activity and promotes MMP-2/MMP-9 mRNA decay by inducing ERK inactivation and p38 MAPK/JNK activation [56]. Downregulation of caffeine inducing MMP-2 and MMP-9 in human leukemia U937 cells was demonstrated by Liu et al. [57]. The mechanism was shown as p38 MAPK/c-Jun pathway activated and ERK/c-Fos pathway suppressed *via* Ca(2+)/ROS [57].

Research reported gallic acid–induced MMP-2/MMP-9 downregulation in human leukemia K562 cells, namely Bcr/Abl-positive cells [58]. Concerning the signaling pathways associated with gallic acid–suppressed invasion of leukemia K562 cells, gallic acid evoked Bcr/Abl degradation in K562 cells through the suppression of c-Jun/ATF-2 and c-Jun/c-Fos pathways; however, Bcr/Abl overexpression attenuated gallic acid–induced MMP-2 and MMP-9 downregulations [58].

## 5 Future research

Several MMPs have been identified to be involved in the growth, migration, invasion, angiogenesis, and apoptosis of leukemic cells, thus predominantly contributing to the degradative capacity of such cells [36]. The prognosis of pediatric patients with relapsed ALL is poor [8]. Molecular pathways implicated in relapse include cell cycle, epigenetic regulation, RAS, JAK STAT, glucocorticoid response, B cell development, nucleotide metabolism, and DNA repair [8]. Hence, therapeutic strategies targeting these pathways constitute a rational medical intervention to prevent relapse [8].

Drugs targeting MMPs have been developed for treating hematological malignancies [40]; MMPs and TIMPs individually or in combination may play a crucial role in basic cellular biology [40]. Several clinical trials have shown negative regulators of tumor growth and metastasis to decrease cancer progression [62–64]. Based on focused targeting of specific MMPs, achieving a balance between required activity and toxicity avoidance is imperative [62]. Preclinical development of metalloproteinase inhibitors, the design of clinical trials, and assessment of clinical response should be addressed [63]. Moreover, studies can be conducted on the application of MMP inhibitors in cancer therapy, such as collagen peptidomimetics, nonpeptidomimetic inhibitors of MMP active sites, bisphosphonates, and tetracycline derivatives [64]. Additional clinical trial design strategies are also required [62–64].

## 6 Conclusion

Leukemia is the most common pediatric cancer, and its incidence has increased over the past decades [65]. A greater understanding of the pathological mechanisms of childhood leukemia is necessary for the development of effective therapeutic strategies. MMPs should be used as therapeutic targets because of their crucial role in leukemia progression. Additional clinical trials are required to establish the association between MMP expression and leukemia prognosis. However, several challenges are involved in routine assessment of MMPs in laboratories, including the heterogeneity and effects of genetic backgrounds of individuals on MMP expression, varied secretion levels in different leukemia types, and the absence of a distinctive pattern of MMP expression in leukemia [36]. Future research should identify the underlying mechanisms and role of MMP expression, role of selective MMP inhibitors in treatment, and toxicity of MMP-targeting drugs through clinical trials involving human models in order to evaluate the potential of MMP inhibitors in the prevention and treatment of childhood leukemia.

## Compliance with ethical standards

**Competing interests** The authors declare that they have no competing interests.

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