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REVIEW

# The advances of methotrexate resistance in rheumatoid arthritis

Jun Yu[1] · Peng Zhou[2]

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**Abstract**
Rheumatoid arthritis (RA) is a systemic autoimmune disease, which is characterized by a chronic fluctuating course and immune dysfunction, resulting in affecting the health and life quality of RA patients. Methotrexate (MTX), as the standard gold treatment of RA, has received more and more clinical applications and basic pharmacological research. In several observational studies, MTXR, and treatment responses in RA patients show that the ratio of MTXR and non- response is about 30%–50%, namely MTX resistance (MTXR). Extensive efforts have been made into the investigation of the mechanism and effective biomarkers in MTXR of RA. In this paper, we discuss the recent findings regarding the critical signaling pathways of MTXR in RA. Provide research targets and directions for a drug therapy that develop preventive strategies and effective treatments of MTXR.

**Keywords** Rheumatoid arthritis · Methotrexate resistance · Mechanism · Response

## Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease, which is characterized by a chronic fluctuating course and immune dysfunction, resulting in progressive joint erosion, deformity, and disability. The prominent clinical manifestation of RA is multiple joints arthritis. The joints such as the hands, wrists, and feet are most commonly affected. Redness, swelling, heat, pain, and dysfunction appear early, and erosive deformities can perform at a later stage. It is a disease with a high disability rate (Ostrowska et al. 2018). The current clinical drugs used to treat RA include nonsteroidal anti-inflammatory drugs (NSAIDs), steroidal anti-inflammatory drugs (SAIDs), and disease-modifying antirheumatic drugs (DMARDs). Still, the long-term application will cause various and even severe adverse reactions (Felson 2016).

Methotrexate (MTX) is folic acid (folate) inhibitor that was first synthesized in the 1940s to treat childhood leukemia. It is extensively used in acute leukemia, breast cancer, and lung cancer. Research in the 1980s found that low-dose MTX can treat RA and has good therapeutic effects, and has gradually become a standard gold treatment for RA (Boerbooms et al. 1995). The American College of Rheumatology (ACR) in 2015, European League Against Rheumatism (EULAR) in 2019, and the Chinese Rheumatology Association in 2018 suggested that the patient is diagnosed with RA, MTX monotherapy is recommended. For patients with middle or high degree disease activity, MTX combination therapy should be considered (Association 2018; Singh et al. 2016; Smolen et al. 2020). In long-term applications, MTX also can cause serious side effects, such as liver damage, gastrointestinal reactions, and bone marrow suppression (Lampropoulos et al. 2015).

Although MTX has a well therapeutic effect on the treatment of RA, about 30%–50% of patients will have nonresponse after taking MTX, or the efficacy was reduced after re-treatment after relapse, that is, MTX resistance (MTXR) (Inoue and Yuasa 2014). MTXR has been extensively researched in the field of anti-tumor therapy and is closely related to multidrug resistance (MDR) (Volk et al. 2000). Compare with the area of anti-tumor, and it is little reported on RA. Although several inflammatory cytokines, drug transporters, and other factors have been researched,

✉ Peng Zhou
zhoupeng@ahtcm.edu.cn

[1] The Fourth Affiliated Hospital of Anhui Medical University, Hefei 230012, China

[2] School of Integrated Chinese and Western Medicine, Anhui Province Key Laboratory of Chinese Medicinal Formula, Anhui University of Chinese Medicine, Institute of Integrated Chinese and Western Medicine, Anhui Academy of Chinese Medicine, Hefei 230012, China

the mechanisms and processes are still being investigated. In this paper, we review the recent findings regarding the signaling pathways of MTXR in RA. Provide research targets and directions for a drug therapy that alleviates the mechanism of MTXR.

## Signaling pathways of MTXR in RA

### Methotrexate mechanism

Folates are an essential substance in the synthesis of purines, pyrimidines, and amino acids, and play a crucial role in the biosynthesis of DNA. As an antifolate, MTX mainly inhibits its dihydrofolate reductase (DHFR), thymidylate synthetase (TYMS), aminoimidazole carboxamide ribonucleotide transformylase (ATIC), resulting in the inhibition of DNA biosynthesis and elevation of extracellular adenosine (Inoue and Yuasa 2014). Previous evidence showed that MTX enters the cell through reduced folate carrier (RCF), and is transformed into MTX polyglutamates (MTX-PGs) under the action of PGs enzyme, thereby generating the effect of inhibiting the enzymes of DHFR, TYMS and ATIC (Leclerc et al. 2010; Wojtuszkiewicz et al. 2015). Besides, MTX-PG inhibits its ATIC enzyme activity more than MTX, deducing that MTX-PGs is the active form of MTX (Dervieux et al. 2004). It should be noted that the conversion of MTX to MTX-PGs is reversible, in which this reaction requires the participation of a gamma-glutamyl hydrolase (GGH) enzyme (Jekic et al. 2013).

DHFR is a mediated enzyme for nucleic acid biosynthesis, which catalyzes the transform of dihydrofolate (DHF) to tetrahydrofolate (THF) (Fig. 1) (Banerjee et al. 2002). In this process, the two molecules of $H^+$ from DHF through the transmit way of NADPH to $NADP^+$, and then enter the $C_6$ atom of the DHF pteridine ring (Li et al. 2018). Then, the THF is converted to methyltetrahydrofolate (MTHF), which is a substrate of TYMS for thymidine biosynthesis (Banerjee et al. 2002). TYMS catalyst is the first phase of DNA synthesis. The DNA synthesis precursor deoxythymidine monophosphate (dTMP) is transformed from deoxyuridine monophosphate (dUMP) (Rose et al. 2002). In this step, MTHF is oxidized to DHF. ATIC is an essential enzyme in the IMP synthesis step. The inhibition of ATIC leads to an increase in aminoimidazole carboxamide ribonucleotide (ATICR), and the inosine monophosphate (IMP) synthesis is inhibited. Inhibition of IMP leads to the further production of adenosine monophosphate (AMP) and guanosine monophosphate (GMP) that eventually reduce the DNA biosynthesis and increase the adenosine (Dervieux et al. 2009; Rayl et al. 1996). Adenosine is involved in many inflammatory cytokine responses and signaling pathways. In general, MTX inhibits folate-dependent enzymes, resulting in blocked DNA synthesis and increased extracellular adenosine levels (Fig. 2).

### ATP binding cassette (ABC) transporters

ABC superfamily is a general term with a variety of transmembrane transporters. Its mechanism is the combination of exogenous substances and specific binding sites on the substrate of transporters. The derived material is pumped out of the cell to maintain the intracellular drug concentration at a certain level. The energy of transport is from the hydrolysis of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) (Priess et al. 2018). ABC transporters that take part

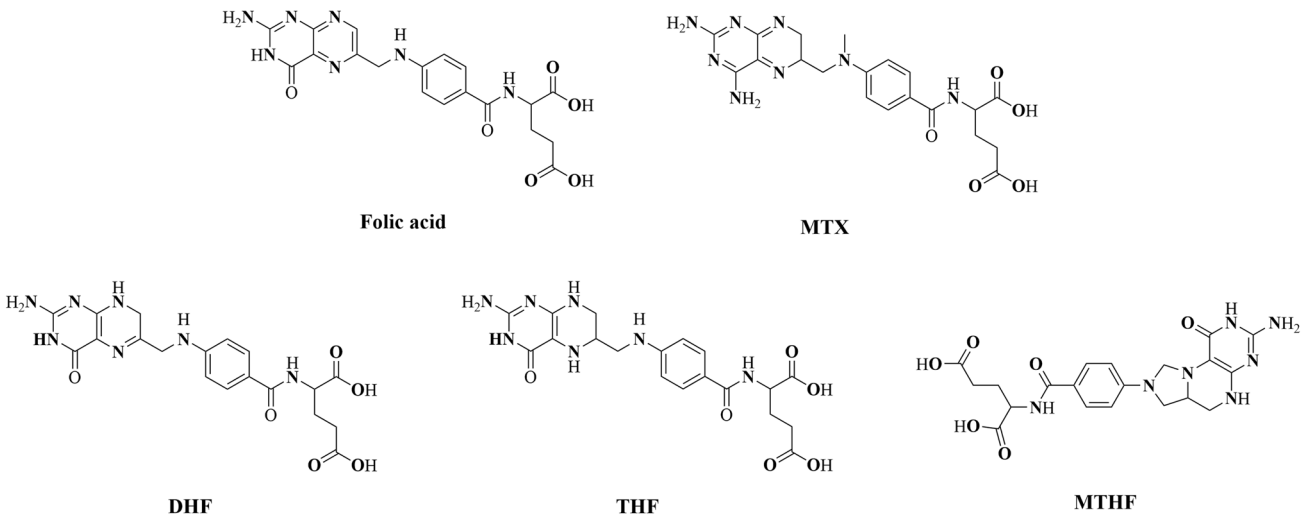


**Fig. 1** The structure of folate, MTX, DHF, THF, and MTHF. *MTX* methotrexate, *DHF* dihydrofolate, *THF* tetrahydrofolate, *MTHF* methyltetrahydrofolate

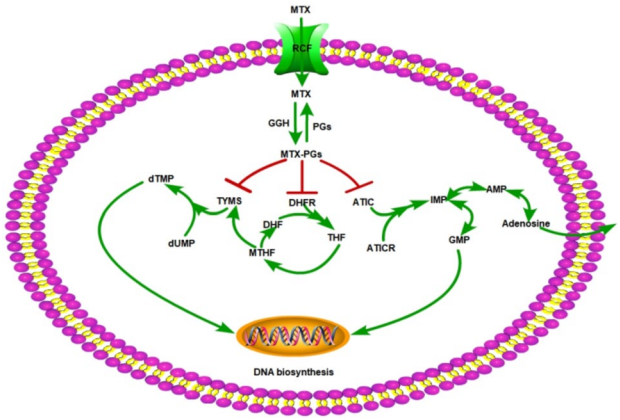


**Fig. 2** Methotrexate mechanism. *MTX* bmethotrexate, *RCF* reduced folate carrier, *GGH* gamma-glutamyl hydrolase, *MTX-PGs* methotrexate polyglutamates, *DHFR* dihydrofolate reductase, *TYMS* thymidylate synthetase, *ATIC* aminoimidazole carboxamide ribonucleotide transformylase, *ATICR* aminoimidazole carboxamide ribonucleotide, *DHF* dihydrofolate, *THF* tetrahydrofolate, *MTHF* methyltetrahydrofolate, *dTMP* deoxythymidine monophosphate, *dUMP* deoxyuridine monophosphate, *IMP* inosine monophosphate, *AMP* adenosine monophosphate, *GMP* guanosine monophosphate

in drug resistance include ABCB1, ABCC1-7, ABCC10/11, ABCG2.

P-glycoprotein (P-gp) (also name ABCB1/multidrug resistance 1 (MDR1)) is a widely researched and essential transporter in the ABC family. It is extensive distribution in the body tissues, mainly in the synovium, spleen, liver, proximal tubules, and mesangial cells of the kidney, apical shallow columnar epithelial cells of the small intestine, *etc*. (Yu et al. 2016). Research finds P-gp linked to MTXR in RA. The expression levels of P-gp in fibroblast-like synoviocytes (FLS) of RA patients are increasing. Besides, the level of P-gp in peripheral blood mononuclear cells (PBMC) and peripheral lymphocytes is significantly higher than the healthy people. The expression level of P-gp in patients with refractory RA is significantly higher than that in non-refractory patients (Liu et al. 2016; Tsujimura and Tanaka 2015). In FLS of RA patients, the high expression of MDR gene MDR-1 is through the up-regulation of P-gp to promote FLS efflux ability to MTX, resulting in MTXR (Wang et al. 2019). In PBMC of MTXR patients, MDR1 mRNA expression also increased, and P-gp efflux function was enhanced, suggesting that the high expression of MDR1/P-gp in RA patients is closely related to MTXR (Yao et al. 2017). Recent studies have found that the increased expression of P-gp in the serum of RA patients is associated with the failure rate of RA treatment (Perez-Guerrero et al. 2018). The high expression of MDR1/P-gp in RA patients may be a potential mechanism for MTXR and the treatment of RA.

The human breast cancer resistance protein (BCRP) is the second member of the ABC efflux transporter superfamily and also named as ABCG2 (Ikemura et al. 2019). Like the function of P-gp, BCRP can make intracellular MTX out of the cell, which is the crucial factor that constitutes the accumulation of MTX in the cell. BCRP expression has been found in the intimal lining layer and on macrophages and endothelial cells in the synovial sublining in RA patients (van der Heijden et al. 2009). Further study found that BCRP expression was significantly increased (three-fold) to non-responders with MTX treatment. This means that BCRP may contribute to the reduced therapeutic efficacy of MTX treatment. Also, Stamp et al. (2013) found that BCRP genes expression involved in the drug transport, and mechanism of MTX are expressed in RA patients' joint synovium. This may influence MTX to play its therapeutic role in the primary site of the RA inflammation process.

The multidrug resistance-associated protein (MRAP) was also one of the important ABC superfamilies and called as ABCC. MRAP contains multiple subtypes involved in MDR, such as MRAP1-MRAP9 (Wu et al. 2018). In the detection of tissue samples of RA patients, it was found that MRAP-1 was lowly expressed on synovial macrophages and moderately expressed in T cells (van der Heijden et al. 2009). MTX is the substrate of MRAP and BCRP, and it is easily affected by transport proteins when entering the body (Micsik et al. 2015). Wolf et al. (2005) reported that the lack of both MRAP and RFC led to a significantly better MTX therapeutic outcome. These together demonstrate the importance of MRAP in MTXR appears.

Conclusively, the ABC superfamily transporters play an essential role in MTXR occurrence (Fig. 3). Determination of these transporters' proteins may predict treatment to MTX, providing further insights into the mechanisms responsible for MTR in RA.

## Transforming growth factor-β (TGF-β)/Smad pathway

TGF-β belongs to the TGF superfamily that regulates cell growth and differentiation. In addition to TGF-βs, this family also includes agonists, inhibitors, critical inhibitor substances, and bone morphogenetic proteins (Sun et al. 2019). Smads are downstream proteins involved in TGF-β-mediated intracellular signaling. At present, nine kinds of Smad proteins can be isolated from the human body, which can be divided into three subtypes according to their functions: Receptor activated Smad (R-Smad), coopartility Smad and inhibitory Smad. Smad2, Smad3, Smad4 and Smad7 are the main participants in the signal transduction pathway mediated by TGF-β from the cell membrane to the nucleus (Massague and Wotton 2000).

In the above parts of this review, clusters of differentiation 39 (CD39) mediate AMP hydrolysis to adenosine, and subsequently, adenosine binds to its receptor and activates

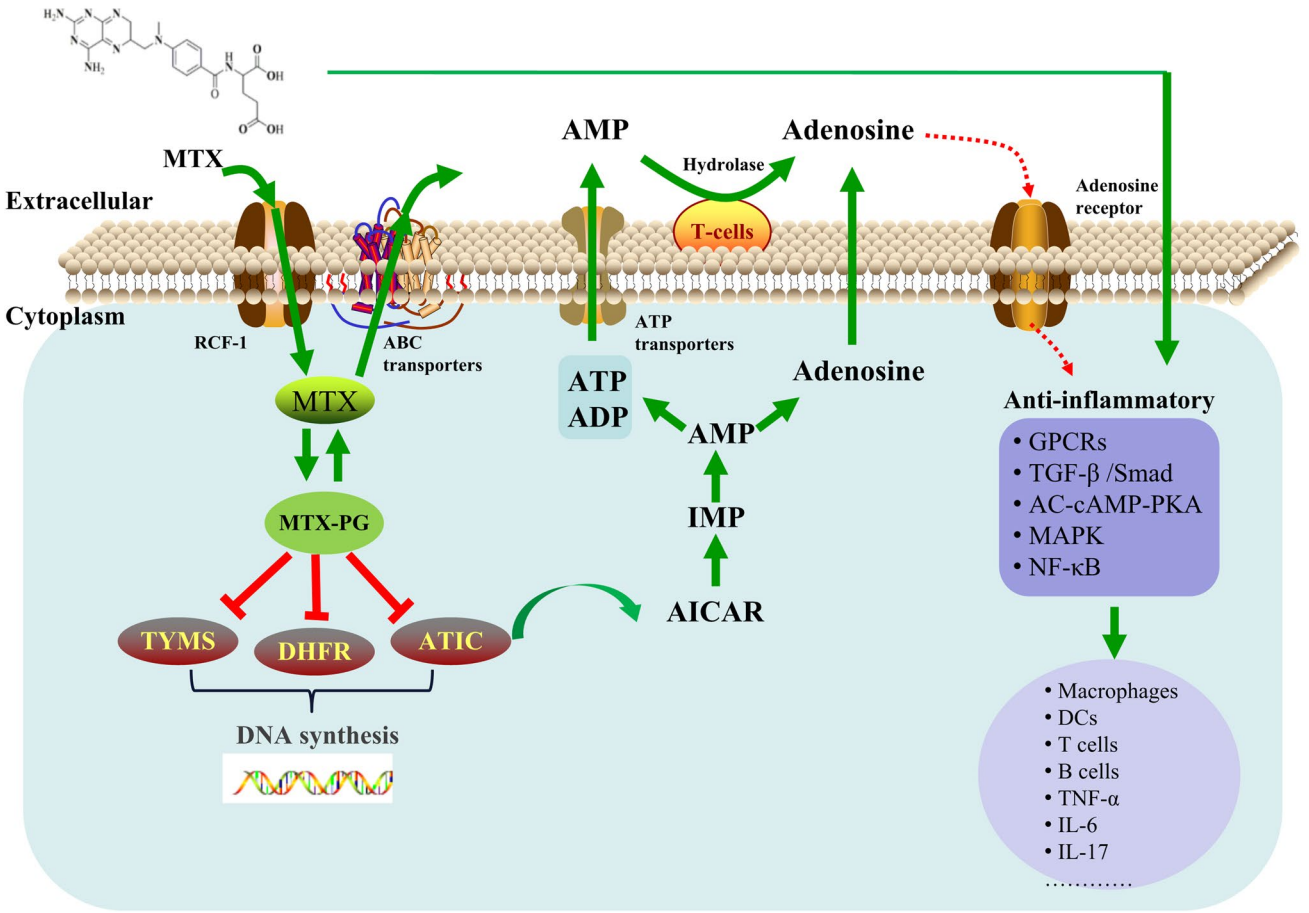


**Fig. 3** The ABC transporters and AR signaling pathways of MTXR in RA. *ABC* ATP-binding cassette, *AR* adenosine receptor, *MTX* methotrexate, *RCF* reduced folate carrier, *MTXR* methotrexate resistance, *RCF* reduced folate carrier, *DHFR* dihydrofolate reductase, *TYMS* thymidylate synthetase, *ATIC* aminoimidazole carboxamide ribonucleotide transformylase, *ATICR* aminoimidazole carboxamide ribonucleotide, *IMP* inosine monophosphate, *AMP* adenosine monophosphate, *ATP* adenosine triphosphate, *ADP* adenosine diphosphate, *GPCRs* G protein-coupled receptors, *TGF-β* transforming growth factor-β, *AC-cAMP-PKA* adenylate cyclase- cyclic adenosine monophosphate-protein kinase A, *MAPK* mitogen-activated protein kinases, *NF-κB* nuclear factor kappa B, *DCs* dendritic cells, *TNF-α* tumor necrosis factor α, *IL* interleukin

downstream signaling pathways (Peres et al. 2015). Regulatory T cells (Tregs) are a subset of T cells critical for immune homeostasis, preventing the onset of autoimmunity disease. The anti-inflammatory effect of MTX is closely related to the expression function of Tregs and the level of extracellular adenosine. TGF-β is a key inducer of Tregs (Shevach et al. 2008). In RA, various factors can activate and phosphorylate TGF-β, and then combine with the TGF-β type II receptor (TGFRII) to form a heterotetrameric complex. The complex binds to and activates the TGF-β type I receptor (TGFRI), and then activated TGFRI links to Smad2, Smad3, and phosphorylates Smad 2, Smad3 to form an oligomer complex, which is finally translocated into the nucleus (Dong et al. 2019; Gao et al. 2020) (Fig. 4). Peres et al. (2015) found that MTXR is associated with low CD39 expression on Tregs and decreased adenosine levels in RA. Further research found that TGF-β induces CD39 expression by increasing ectonucleoside triphosphate diphosphohydrolase-1 (ENTPD1) gene transcription, activation of TGFRII/TGFRI, Smad2 and the transcription factor cAMP response element-binding protein (CREB) (Peres et al. 2018). It is worth noting that this phenomenon shows the specific of Treg, other effector T cells have no such effect. This uniqueness may be related to TGF-induced high expression of Tregs.

Consequently, the CD39 expression could be a biomarker and potential targets for therapeutic intervention for MTXR.

## IL-13$^{+}$CD4$^{+}$ T cell pathway

T-lymphocytes (also known as T-cells) are derived from bone marrow-derived lymphatic stem cells. To perform its immune functions, T-cells distribute from lymphatic and blood circulation into immune organs and tissues (Ma et al.

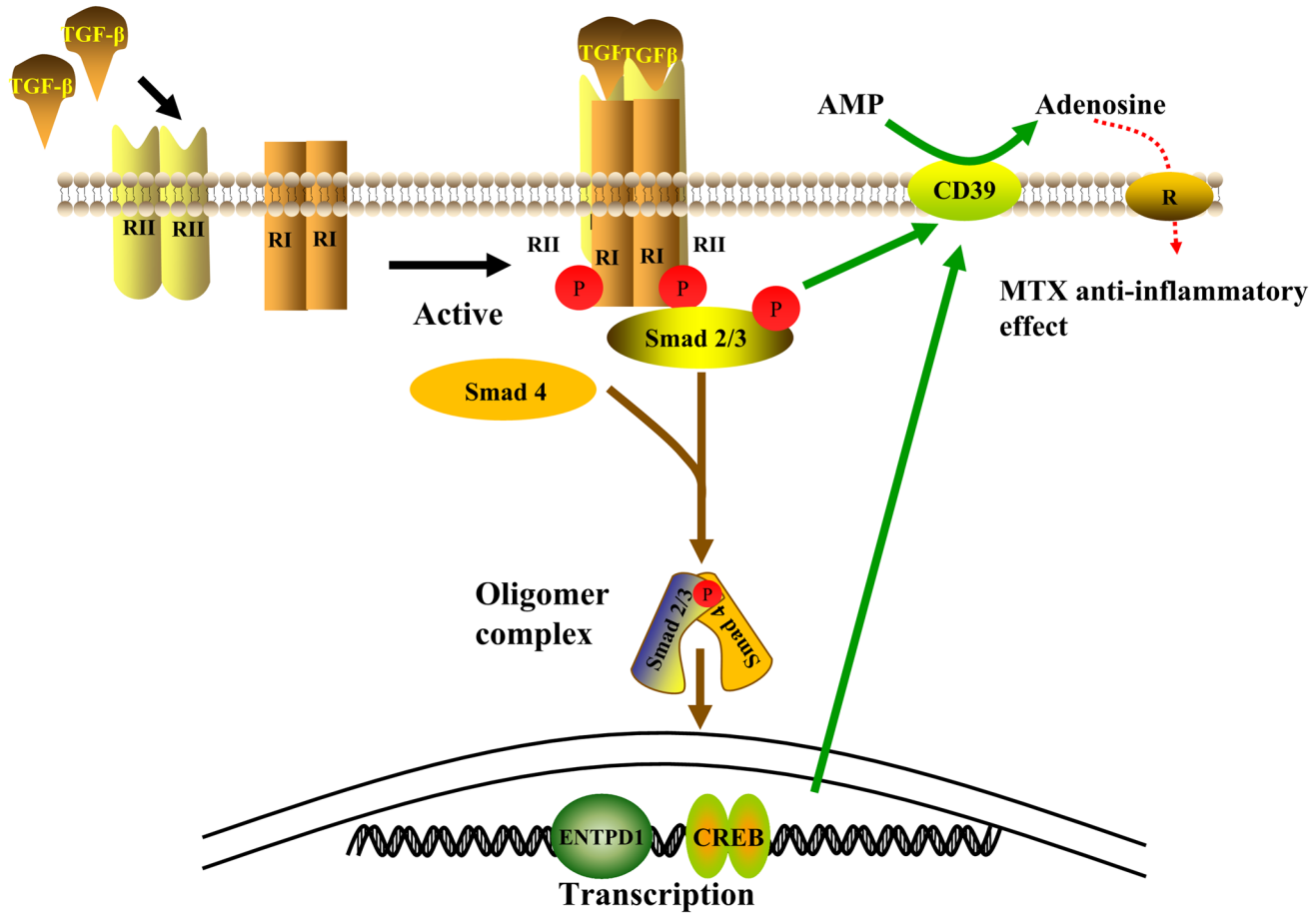


**Fig. 4** The TGF-β/Smad pathway of MTXR in RA. *TGF-β* transforming growth factor-β, *MTXR* methotrexate resistance, *RI* TGF-β type I receptor, *RII* TGF-β type II receptor, *AMP* adenosine monophosphate, *CD39* clusters of differentiation 39, *P* phosphorylation, *R* adenosine receptor, *ENTPD1* ectonucleoside triphosphate diphosphohydrolase-1, *CREB* cAMP response element-binding protein

2019). T-cells are divided into several subgroups, including natural, effector, suppressor, memory, cytotoxic (Tc), regulatory (Tregs) and helper T cells (Th) (Lubberts 2015). Among these, because it expresses on the surface, Th cells are also called $CD4^+$ cells. In the same way, Tc cells are also called $CD8^+$ cells, and so on. T-cells have multiple biological functions. On the one hand, T-cells activate synovial cells through intercellular interaction, which include FLS, macrophages, B-cells, and osteoclasts. On the other hand, T-cells produce immune response and cytokines, including interferon-γ, tumor necrosis factor, interleukin (IL) -6, and IL-17 (Herrath et al. 2011; van Hamburg et al. 2011; Wehrens et al. 2011).

A recent study by Slauenwhite et al. (2020) reported novel biomarker features for MTXR patients. They found that the phenomenon of higher IL-$13^+CD4^+$ T effector memory ($T_{EM}$) frequency, lower CD4: CD8 ratio, higher IL-$13^+$ T cell level in the blood, and higher T cell costimulator-positive ($ICOS^+$) Treg frequency highlight the distinct immunologic phenotypes associated with MTX non-response in RA patients. Baseline CD4: CD8 ratio and IL-$13^+CD4^+T_{EM}$ cell frequency each were associated with the change in disease activity resulting from MTX therapy. That means $CD4^+$ and $CD8^+$ T cells may be playing an essential role in circulating IL-13 in MTXR patients (Dulic et al. 2017). This may be related to different phenotypic expression positions and physiological function. Research shows that CD4 is mainly on the synovium, while CD8 is primarily on the synovial fluid (Verburg et al. 2005). Furthermore, the frequency of $ICOS^+$ Tregs was higher in MTXR patients, and $ICOS^+$ Tregs expressed more IL-17 anti-inflammatory cytokines, which may have a vital role in RA patients with MTX therapy outcome (Wang et al. 2018).

In general, targeting the IL-$13^+CD4^+$T cell pathway could be a new therapeutic strategy in MTXR RA patients. Furthermore, combine this pathway with PBMC and paired synovial fluid data, which may provide a novel research direction for resolving immune-mediated mechanisms of MTXR (Slauenwhite et al. 2020).

## DNA methylation

As mentioned in the MTX mechanism, MTX mainly inhibits DHFR, TYMS, ATIC, resulting in the inhibition of folates, purine, and pyrimidine synthesis, with subsequent blocking of DNA biosynthesis. Likewise, Similarly, MTX may affect other folate-dependent metabolic pathways, such as the methionine regeneration pathway (Stempak et al. 2005). Methionine is essential for the synthesis of S-adenosylmethionine (SAM) and methionine S-adenosyltransferase (MAT), which both are vital in immune-mediated cellular reactions and mainly donor of methyl groups in vivo. There have been studies on the effect of MTX in SAM and MAT in the past. Nesher and Moore (1990) found that MTX inhibits the methionine regeneration, thereby inhibiting SAM and polyamine synthesis. The possible mechanism may be related to the decreased intracellular MTHF of MTX, as a result of DHFR and methylene THFR inhibition (Bunni et al. 1988). In another study, MTX significantly decreased S-adenosylmethionine production by inhibition of MAT independent of folate depletion (Wang and Chiang 2012).

SAM and MAT provide methyl groups for global DNA methylation (Kim et al. 1996). Assuming that the MTX effect on the level of global DNA methylation by inhibiting SAM and MAT. To confirm this hypothesis, the association between the level of global DNA methylation in leukocyte of RA, before-, at 3 months, and over 3 months of MTX therapy with changes in disease activity was raised by Gosselt et al. (2019). They are found a higher baseline global DNA methylation is associated with clinical non-response, determined at 3 months of MTX treatment. This means that global DNA methylation is independently associated with disease activity over the first 3 months of MTX therapy, which could be a prediction marker for MTXR.

There are other few studies on the relationship between global DNA methylation with MTX response. For instance, a study found that the global DNA methylation in peripheral blood cell subpopulations (e.g., T cell, B cell, natural killer (NK) cells, monocytes, and polymorphonuclear leukocytes) was increased by MTX treatment in RA patients. In addition, expression levels of methylation-specific enzymes were also increased in monocytes and T cells (de Andres et al. 2015). In another study, Cribbs et al. (2015) showed Treg function research, which found that the inhibitory function of Treg is defective in untreated RA patients' group. MTX restores defective Treg function through demethylation of the forkhead box P3 (Foxp3) locus that leads to a subsequent increase in Treg gene expression of Foxp3 and cytotoxic T lymphocyte antigen-4 (CTLA-4).

As already mentioned in the research finding, the meaning of global DNA methylation in RA may also act as a biomarker of MTX treatment response (Fig. 5).

## Adenosine recepoter (AR)

As shown in Figs. 1, 2, MTX has an anti-inflammatory effect on RA patients by increasing the level of extracellular adenosine. Adenosine acts on inflammatory cells through AR. AR contains a group of G protein-coupled receptors (GPCRs) that mediate the physiological role of adenosine. So far, four AR subtypes have been cloned and identified in different tissues. These receptors were included ARA1, ARA2A, ARA2B, and ARA3, which have different localizations, signal transduction pathways, and regulatory mechanism (Hasko et al. 2008). One of the most important differences for MTX treatment is that A2A and A2A are associated with Gs protein to induce the production of cyclic AMP (cAMP). However, A1 and A3 are related to Gi protein to inhibit the cAMP (Cronstein and Sitkovsky 2017). Furthermore, compared with the healthy group, ARA2A and ARA3 were up-regulated in lymphocytes of RA patients. A2A and A3AR activation

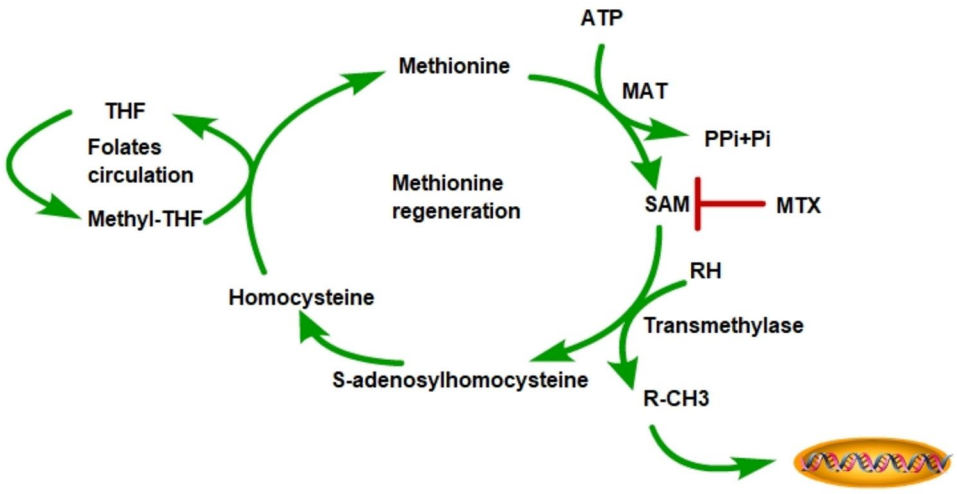


**Fig. 5** The DNA methylation pathway of MTXR in RA. *MTXR* methotrexate resistance, *ATP* adenosine triphosphate, *SAM* S-adenosylmethionine, *MAT* methionine S-adenosyltransferase, *THF* tetrahydrofolate, *PPi* pyrophosphoric acid; *Pi* phosphoric acid

can reduce inflammatory cytokine levels such as TNF-α, IL-1β and IL-6 Varani et al. 2011). Conclusively, AR is a crucial medium of MTX to produce anti-inflammatory effects in RA.

Earlier studies have a focus on the relationship between inflammation and adenosine and MTX treatment. Infusion of ARA2 receptor agonists can significantly enhance bradykinin-induced plasma extravasation (PE) of acute inflammation in the rat knee joint. ARA2 receptor agonists can also reduce arthritis-related joint damage (Green et al. 1991). In vivo of murine air pouch model, after intraperitoneal injection of MTX in model mice for 3-4 weeks. It significantly inhibited the accumulation of leukocyte. The MTX-mediated reduction in leukocyte accumulation was entirely reversed by the ARA2 antagonist, but not affected by ARAl antagonist (Cronstein et al. 1993). This also means that the specificity of the MTX's anti-inflammatory action is receptor-specific.

In addition, Nesher et al. (2003) confirmed that the daily substance of AR antagonists, caffeine, affects the treatment response of MTX in RA patients. Lastly, Montesinos et al. (2006) examined the therapeutic effect of MTX on AR gene knockout mice of the peritoneal inflammation model. They found that MTX treatment increased the concentration of adenosine in the peritoneal exudates of all mice and reduced the accumulation of leukocytes in wild-type mice and ARA3 knockout mice. Additionally, IL-10 levels increased in wild-type mice and ARA3 knockout mice after MTX treatment but decreased in ARA2A knockout mice. These research results also provide strong evidence that different receptors mediate the anti-inflammatory effects of MTX and adenosine in various inflammatory sites. This observation may explain why some inflammatory diseases respond differently to MTX treatment. With continuing this research, Montesinos et al. (2007) found that MTX treatment reduced the levels of leukocytes and tumor necrosis factor-α (TNF-α), increased the concentration of adenosine in wild-type mice. But these phenomena were not found in the experiment of CD73 knockout mice. A similar event was found by Varani et al. (2009). They found that the level of TNF-α in early RA patients is affected by MTX treatment, which is mediated by the nuclear factor kappa B (NFκB) signal pathway. A report of genetic polymorphisms in the adenosine pathway revealed that ATIC C347G polymorphism is inconsistently associated with MTX treatment response. This is due to clinical interventions related to the improvement of ATIC 347CC and GG genotypes (Grabar et al. 2010). The inflammation and clinical response of RA are regulated by AR (Fig. 3). In addition to continuing to study the mechanism of action of MTX on AR, it also supports the use of AR agonists as a new and effective drug for RA patients.

## Other factors

In addition to the above mentioned, other factors affect the occurrence of MTXR. These factors include but not limited to disease period, molecular factors, and patients' factors. Molecular factors, Ally et al. (2015) investigated the correlation between the changes in circulating anti-citrullinated peptide antibodies (ACPA) and inflammatory cytokines (e.g., IL-4, IL-7, IL-8, *etc*.) levels before and after treatment with MTX response in 140 early RA patients. They found that the level of ACPA and pro-inflammatory cytokines were significantly decreased by treating MTX for 6 months. However, the predictive value of continuous measurement of these biomarkers for MTX treatment response, especially related to imaging progress and functional disability, remains to be determined in the future. In another study, low- and medium-concentration pretreatment ACPA correlates with MTX treatment response in ACPA-positive RA patients, which means that quantitative assessment of ACPA levels may be used to determine which patients will benefit most from MTX treatment (Visser et al. 2008).

The same research method can be used for the study of rheumatoid factor (RF), TNF-α, and T-cells subset in MTX treatment response (Bobbio-Pallavicini et al. 2007; Ponchel et al. 2014). TNF-α is a pro-inflammatory cytokine mainly produced by activated macrophages, NK cells, and T-cells. Also, its concentration increases in RA patients (Choy and Panayi 2001). Considering the role of TNF-α in the pathogenesis of RA, the benefit obtained by using MTX is that it may provide sufficient information for predicting anti-MTX response in RA patients. The study found that the TNF-α 308 A/A or A/G genotype of RA patients with the higher biological activity of circulating TNF-α are more sensitive to specific inhibitors (Marotte et al. 2005, 2008; Pachot et al. 2007). Such after systemic treatment with TNF-α specific inhibitor rituximab, TNF-α response production IL-6 was more significant in patients with an excellent clinical response than in the poor responders. Another factor (Ponchel et al. 2014) explored the relationship between disturbance of T-cell subsets and treatment response in patients with early RA treated with MTX or combined anti-TNF therapy. They found that reducing the T-cell baseline is positively correlated with MTX response in early RA patients. This means baseline T-cell subset analysis has a value in predicting early RA patients with first therapy with MTX.

To add more, myeloid-related protein (MRP) belongs to human pro-inflammatory calcium-binding proteins, which are specifically expressed in granulocytes and monocytes (Foell and Roth 2004). The study found that MRP8/14 levels showed a local increase in the inflammation sites and serum of RA patients (Odink et al. 1987; Youssef et al. 1999). A recent study in 87 active RA patients uncovered that after 4 months of MTX treatment, the median interquartile range

baseline level of MRP8/14 was higher of MTX responders compared with MTX non-responders. The Serum MRP level is positively correlated with CRP (Patro et al. 2016).

Patients' factors, many studies have found that the patient's factors will affect the response of MTX treatment. For example, gender, age, psychological factors, disease duration, mood, and lifestyle. Saevarsdottir et al. (2011) use EULAR response criteria to investigate 405 RA patients who took MTX for 3–4 months with symptom duration of less than 1 year, and found that woman MTX treatment response is increased. Furthermore, smoking and the elderly are strongly correlated with MTX response. A survey study of 844 early RA patients within 1 year of onset and 2 years of follow-up, reported that the women showed higher disease activity and joint damage than that in man. However, older women had higher disease severity than those under 50 years of age women (Tengstrand et al. 2004). This sex difference may be related to the effect of estrogen on the immune system. In a study about the association between folate synthase gene polymorphism and MTX response in 281 RA patients, age shows significant differences in good/poor MTX responders groups (Sharma et al. 2009). The results revealed that the average age is 41 years old in MTX poor responders. The good responder's group is 45 years old. This means a specific correlation between MTX's response and age. In another study, Vilca et al. (2010) found that longer disease duration, anti-nuclear antibody negativity, higher disability and presence of wrist activity were significantly associated with a weaker response to MTX treatment with 6 months in 563 RA patients. Mood also affects the MTX treatment response. A recent study of 1326 RA patients with MTX treatment reported that depression and anxiety might affect the likelihood of joint symptoms relief in RA patients (Michelsen et al. 2017). This is a reminder that the patient's emotional state may have specific effects on MTXR, which requires further research. Similarly, a study was to use demographic, clinical and psychosocial variables to predict MTX response in 1050 RA patients, which 43% of them are MTXR patients (Sergeant et al. 2018). They revealed that patient anxiety is a predictor of MTXR and can be noted at the beginning of the patient's treatment.

## Conclusion and perspectives

In summary, this review has discussed the currently recognized mechanism of MTR, and the factors that cause non-responses of MTX treatment in RA. Inflammatory cytokines and drug resistance-related proteins, such as folates synthesis related enzymes, ABC transporters, T-cells, IL-13, IL-4, IL-7, IL-8, TNF-α, SAM, MAT, DNA methylation, AR, ACPA, and MRP, are involved in the occurrence and development of MTXR. Besides, many signaling pathways affect MTXR through molecular signal regulation in RA. These signaling pathways were included TGF-β/Smad pathway, NF-κB pathway, IL-13$^{+}$CD4$^{+}$ T cell pathway, GPCR pathway, and folate-dependent metabolic pathways. It worth noting that RA patients' lifestyle also affects MTXR and treatment response assessment, such as gender, age, psychological factors, disease duration, emotions, lifestyle, and *etc*.

Different signaling pathways, proteins, cytokines, and patient factors promote MTXR production and development through different mechanisms of action. However, these factors never exist alone, but affect the progress of MTXR through multiple contact and influence. Further research should focus on the inter-relationships and cross-expression of MTXR-related signaling pathways, proteins, factors, and patients' state, which is of great significance for revealing the pathogenesis of RA. It is believed that with the deepening of research, a variety of signal pathways, genes, and proteins that have been elucidated will have a positive effect on therapeutic strategies and may relieve the progress of MTXR.

RA is a systemic immune disease, and 20%–30% of untreated RA patients within 3 years will cause permanent loss of function (Ercan et al. 2010; Wei 2016). Erosive malformations can occur in the late stage of RA, a disease with a high disability rate, which seriously affects the health and quality of life of RA patients (Brown et al. 2017). MTX, as the gold standard treatment of RA, has received more and more clinical applications and basic pharmacological research. In many observational studies, MTXR and therapy responses in RA patients in the current era were investigated. The results show that the ratio of MTXR and non- response is about 30% –50% (Vilca et al. 2010; Braun et al. 2008; Mori et al. 2010; van Vollenhoven et al. 2009). Research to find appropriate RA treatment drugs combined with MTX is the current research focus. Despite the individual variation, the mechanism of MTXR and the degree of treatment response needs further and thorough study. Extensive efforts have been made into the investigation of useful biomarkers to assist the clinical judgment of the efficacy of drugs in the early RA diagnosis or drug treatment (Halilova et al. 2012; Yu et al. 2018). However, at present, this field is still a great challenge because there are no reliable, authoritative studies that can be used to predict MTX response clinically. In future investigations and research, the representative sample size, high-throughput technologies (such as proteomics, transcriptomics, and systematic genome sequencing), epigenetic factors, and comprehensive multidisciplinary research should be considered (Selga et al. 2009; Wong et al. 2017).

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no competing interests.

## References


Ally MM, Hodkinson B, Meyer PW, Musenge E, Tintinger GR, Tikly M, Anderson R (2015) Circulating anti-citrullinated peptide antibodies, cytokines and genotype as biomarkers of response to disease-modifying antirheumatic drug therapy in early rheumatoid arthritis. BMC Musculoskelet Disord 16:130. https://doi.org/10.1186/s12891-015-0587-1

Association CR (2018) 2018 Chinese guideline for the diagnosis and treatment of rheumatoid arthritis. Chin J Intern Med 57:242–251

Banerjee D, Mayer-Kuckuk P, Capiaux G, Budak-Alpdogan T, Gorlick R, Bertino JR (2002) Novel aspects of resistance to drugs targeted to dihydrofolate reductase and thymidylate synthase. Biochim Biophys Acta 1587:164–173. https://doi.org/10.1016/s0925-4439(02)00079-0

Bobbio-Pallavicini F, Caporali R, Alpini C, Moratti R, Montecucco C (2007) Predictive value of antibodies to citrullinated peptides and rheumatoid factors in anti-TNF-alpha treated patients. Ann N Y Acad Sci 1109:287–295. https://doi.org/10.1196/annals.1398.034

Boerbooms AM, Kerstens PJ, van Loenhout JW, Mulder J, van de Putte LB (1995) Infections during low-dose methotrexate treatment in rheumatoid arthritis. Semin Arthritis Rheum 24:411–421. https://doi.org/10.1016/s0049-0172(95)80009-3

Braun J et al (2008) Comparison of the clinical efficacy and safety of subcutaneous versus oral administration of methotrexate in patients with active rheumatoid arthritis: results of a six-month, multicenter, randomized, double-blind, controlled, phase IV trial. Arthr Rheum 58:73–81. https://doi.org/10.1002/art.23144

Brown JM et al (2017) Detection and characterisation of bone destruction in murine rheumatoid arthritis using statistical shape models. Med Image Anal 40:30–43. https://doi.org/10.1016/j.media.2017.05.006

Bunni M, Doig MT, Donato H, Kesavan V, Priest DG (1988) Role of methylenetetrahydrofolate depletion in methotrexate-mediated intracellular thymidylate synthesis inhibition in cultured L1210 cells. Cancer Res 48:3398–3404

Choy EH, Panayi GS (2001) Cytokine pathways and joint inflammation in rheumatoid arthritis. N Engl J Med 344:907–916. https://doi.org/10.1056/NEJM200103223441207

Cribbs AP et al (2015) Methotrexate restores regulatory T cell function through demethylation of the FoxP3 upstream enhancer in patients with rheumatoid arthritis. Arthritis Rheumatol 67:1182–1192. https://doi.org/10.1002/art.39031

Cronstein BN, Sitkovsky M (2017) Adenosine and adenosine receptors in the pathogenesis and treatment of rheumatic diseases. Nat Rev Rheumatol 13:41–51. https://doi.org/10.1038/nrrheum.2016.178

Cronstein BN, Naime D, Ostad E (1993) The antiinflammatory mechanism of methotrexate. Increased adenosine release at inflamed sites diminishes leukocyte accumulation in an in vivo model of inflammation. J Clin Invest 92:2675–2682. https://doi.org/10.1172/JCI116884

de Andres MC, Perez-Pampin E, Calaza M, Santaclara FJ, Ortea I, Gomez-Reino JJ, Gonzalez A (2015) Assessment of global DNA methylation in peripheral blood cell subpopulations of early rheumatoid arthritis before and after methotrexate. Arthritis Res Ther 17:233. https://doi.org/10.1186/s13075-015-0748-5

Dervieux T, Furst D, Lein DO, Capps R, Smith K, Walsh M, Kremer J (2004) Polyglutamation of methotrexate with common polymorphisms in reduced folate carrier, aminoimidazole carboxamide ribonucleotide transformylase, and thymidylate synthase are associated with methotrexate effects in rheumatoid arthritis. Arthritis Rheum 50:2766–2774. https://doi.org/10.1002/art.20460

Dervieux T, Wessels JA, van der Straaten T, Penrod N, Moore JH, Guchelaar HJ, Kremer JM (2009) Gene-gene interactions in folate and adenosine biosynthesis pathways affect methotrexate efficacy and tolerability in rheumatoid arthritis. Pharmacogenet Genom 19:935–944. https://doi.org/10.1097/FPC.0b013e32833315d1

Dong X, Gan Y, Ding L, Zeng F, Ding D (2019) Effect of jiawei fengshining on synovial cell apoptosis and TGF-beta1/Smad signaling pathway in rats with rheumatoid arthritis evid based complement. Alternat Med 2019:8614034. https://doi.org/10.1155/2019/8614034

Dulic S et al (2017) T-cell subsets in rheumatoid arthritis patients on long-term anti-TNF or IL-6 receptor blocker therapy. Mediat Inflamm 2017:6894374. https://doi.org/10.1155/2017/6894374

Ercan A et al (2010) Aberrant IgG galactosylation precedes disease onset, correlates with disease activity, and is prevalent in autoantibodies in rheumatoid arthritis. Arthritis Rheum 62:2239–2248. https://doi.org/10.1002/art.27533

Felson DT (2016) Safety of nonsteroidal antiinflammatory drugs. N Engl J Med 375:2595–2596. https://doi.org/10.1056/NEJMe1614257

Foell D, Roth J (2004) Proinflammatory S100 proteins in arthritis and autoimmune disease. Arthritis Rheum 50:3762–3771. https://doi.org/10.1002/art.20631

Gao M, Zheng J, Zheng C, Huang Z, Huang Q (2020) Theacrine alleviates chronic inflammation by enhancing TGF-beta-mediated shifts via TGF-beta/SMAD pathway in Freund's incomplete adjuvant-induced rats. Biochem Biophys Res Commun 522:743–748. https://doi.org/10.1016/j.bbrc.2019.11.126

Gosselt HR, van Zelst BD, de Rotte M, Hazes JMW, de Jonge R, Heil SG (2019) Higher baseline global leukocyte DNA methylation is associated with MTX non-response in early RA patients. Arthritis Res Ther 21:157. https://doi.org/10.1186/s13075-019-1936-5

Grabar PB, Rojko S, Logar D, Dolzan V (2010) Genetic determinants of methotrexate treatment in rheumatoid arthritis patients: a study of polymorphisms in the adenosine pathway. Ann Rheum Dis 69:931–932. https://doi.org/10.1136/ard.2009.111567

Green PG, Basbaum AI, Helms C, Levine JD (1991) Purinergic regulation of bradykinin-induced plasma extravasation and adjuvant-induced arthritis in the rat. Proc Natl Acad Sci USA 88:4162–4165. https://doi.org/10.1073/pnas.88.10.4162

Halilova KI, Brown EE, Morgan SL, Bridges SL Jr, Hwang MH, Arnett DK, Danila MI (2012) Markers of treatment response to methotrexate in rheumatoid arthritis: where do we stand? Int J Rheumatol 2012:978396. https://doi.org/10.1155/2012/978396

Hasko G, Linden J, Cronstein B, Pacher P (2008) Adenosine receptors: therapeutic aspects for inflammatory and immune diseases. Nat Rev Drug Discov 7:759–770. https://doi.org/10.1038/nrd2638

Herrath J et al (2011) The inflammatory milieu in the rheumatic joint reduces regulatory T-cell function. Eur J Immunol 41:2279–2290. https://doi.org/10.1002/eji.201041004

Ikemura K et al (2019) Concomitant febuxostat enhances methotrexate-induced hepatotoxicity by inhibiting breast cancer resistance protein. Sci Rep 9:20359. https://doi.org/10.1038/s41598-019-56900-2

Inoue K, Yuasa H (2014) Molecular basis for pharmacokinetics and pharmacodynamics of methotrexate in rheumatoid arthritis therapy. Drug Metab Pharmacokinet 29:12–19. https://doi.org/10.2133/dmpk.dmpk-13-rv-119

Jekic B et al (2013) Association of the TYMS 3G/3G genotype with poor response and GGH 354GG genotype with the bone marrow toxicity of the methotrexate in RA patients. Eur J Clin Pharmacol 69:377–383. https://doi.org/10.1007/s00228-012-1341-3

Kim YI, Logan JW, Mason JB, Roubenoff R (1996) DNA hypomethylation in inflammatory arthritis: reversal with methotrexate. J Lab Clin Med 128:165–172. https://doi.org/10.1016/s0022-2143(96)90008-6

Lampropoulos CE et al (2015) Adverse events and infections in patients with rheumatoid arthritis treated with conventional drugs or biologic agents: a real world study. Clin Exp Rheumatol 33:216–224

Leclerc GJ, Mou C, Leclerc GM, Mian AM, Barredo JC (2010) Histone deacetylase inhibitors induce FPGS mRNA expression and intracellular accumulation of long-chain methotrexate polyglutamates in childhood acute lymphoblastic leukemia: implications for combination therapy. Leukemia 24:552–562. https://doi.org/10.1038/leu.2009.282

Li H et al (2018) Inhibitors of dihydrofolate reductase as antitumor agents: design, synthesis and biological evaluation of a series of novel nonclassical 6-substituted pyrido[3,2-d]pyrimidines with a three- to five-carbon bridge. Bioorg Med Chem 26:2674–2685. https://doi.org/10.1016/j.bmc.2018.04.035

Liu YM, Chen JW, Chen LX, Xie X, Mao N (2016) Overexpression of P-glycoprotein on fibroblast-like synoviocytes in refractory rheumatoid arthritis patients: a potential mechanism for multidrug resistance in rheumatoid arthritis treatment. Genet Mol Res. https://doi.org/10.4238/gmr.15027927

Lubberts E (2015) Role of T lymphocytes in the development of rheumatoid arthritis. Implications for treatment. Curr Pharm Des 21:142–146. https://doi.org/10.2174/1381612820666140825122247

Ma D et al (2019) Immunomodulatory effect of human umbilical cord mesenchymal stem cells on T lymphocytes in rheumatoid arthritis. Int Immunopharmacol 74:105687. https://doi.org/10.1016/j.intimp.2019.105687

Marotte H, Maslinski W, Miossec P (2005) Circulating tumour necrosis factor-alpha bioactivity in rheumatoid arthritis patients treated with infliximab: link to clinical response. Arthritis Res Ther 7:R149–155. https://doi.org/10.1186/ar1465

Marotte H, Arnaud B, Diasparra J, Zrioual S, Miossec P (2008) Association between the level of circulating bioactive tumor necrosis factor alpha and the tumor necrosis factor alpha gene polymorphism at -308 in patients with rheumatoid arthritis treated with a tumor necrosis factor alpha inhibitor. Arthritis Rheum 58:1258–1263. https://doi.org/10.1002/art.23430

Massague J, Wotton D (2000) Transcriptional control by the TGF-beta/Smad signaling system. EMBO J 19:1745–1754. https://doi.org/10.1093/emboj/19.8.1745

Michelsen B et al (2017) Do depression and anxiety reduce the likelihood of remission in rheumatoid arthritis and psoriatic arthritis? Data from the prospective multicentre NOR-DMARD study. Ann Rheum Dis 76:1906–1910. https://doi.org/10.1136/annrheumdis-2017-211284

Micsik T, Lorincz A, Gal J, Schwab R, Petak I (2015) MDR-1 and MRP-1 activity in peripheral blood leukocytes of rheumatoid arthritis patients. Diagn Pathol 10:216. https://doi.org/10.1186/s13000-015-0447-1

Montesinos MC, Desai A, Cronstein BN (2006) Suppression of inflammation by low-dose methotrexate is mediated by adenosine A2A receptor but not A3 receptor activation in thioglycollate-induced peritonitis. Arthritis Res Ther 8:R53. https://doi.org/10.1186/ar1914

Montesinos MC, Takedachi M, Thompson LF, Wilder TF, Fernandez P, Cronstein BN (2007) The antiinflammatory mechanism of methotrexate depends on extracellular conversion of adenine nucleotides to adenosine by ecto-5′-nucleotidase: findings in a study of ecto-5′-nucleotidase gene-deficient mice. Arthritis Rheum 56:1440–1445. https://doi.org/10.1002/art.22643

Mori S, Hirose J, Yonemura K (2010) Contribution of HLA-DRB1*04 alleles and anti-cyclic citrullinated antibodies to development of resistance to disease-modifying antirheumatic drugs in early rheumatoid arthritis. Clin Rheumatol 29:1357–1366. https://doi.org/10.1007/s10067-010-1454-y

Nesher G, Moore TL (1990) The in vitro effects of methotrexate on peripheral blood mononuclear cells. Modulation by methyl donors and spermidine. Arthritis Rheum 33:954–959. https://doi.org/10.1002/art.1780330706

Nesher G, Mates M, Zevin S (2003) Effect of caffeine consumption on efficacy of methotrexate in rheumatoid arthritis. Arthritis Rheum 48:571–572. https://doi.org/10.1002/art.10766

Odink K et al (1987) Two calcium-binding proteins in infiltrate macrophages of rheumatoid arthritis. Nature 330:80–82. https://doi.org/10.1038/330080a0

Ostrowska M, Maslinski W, Prochorec-Sobieszek M, Nieciecki M, Sudol-Szopinska I (2018) Cartilage and bone damage in rheumatoid arthritis. Reumatologia 56:111–120. https://doi.org/10.5114/reum.2018.75523

Pachot A et al (2007) Increased tumor necrosis factor-alpha mRNA expression in whole blood from patients with rheumatoid arthritis: reduction after infliximab treatment does not predict response. J Rheumatol 34:2158–2161

Patro PS, Singh A, Misra R, Aggarwal A (2016) Myeloid-related protein 8/14 levels in rheumatoid arthritis: marker of disease activity and response to methotrexate. J Rheumatol 43:731–737. https://doi.org/10.3899/jrheum.150998

Peres RS et al (2015) Low expression of CD39 on regulatory T cells as a biomarker for resistance to methotrexate therapy in rheumatoid arthritis. Proc Natl Acad Sci USA 112:2509–2514. https://doi.org/10.1073/pnas.1424792112

Peres RS et al (2018) TGF-beta signalling defect is linked to low CD39 expression on regulatory T cells and methotrexate resistance in rheumatoid arthritis. J Autoimmun 90:49–58. https://doi.org/10.1016/j.jaut.2018.01.004

Perez-Guerrero EE et al (2018) Serum P-glycoprotein level: a potential biomarker of DMARD failure in patients with rheumatoid arthritis. Inflammopharmacology. https://doi.org/10.1007/s10787-018-0529-2

Ponchel F et al (2014) An immunological biomarker to predict MTX response in early RA. Ann Rheum Dis 73:2047–2053. https://doi.org/10.1136/annrheumdis-2013-203566

Priess M, Goddeke H, Groenhof G, Schafer LV (2018) Molecular mechanism of ATP hydrolysis in an ABC transporter. ACS Cent Sci 4:1334–1343. https://doi.org/10.1021/acscentsci.8b00369

Rayl EA, Moroson BA, Beardsley GP (1996) The human purH gene product, 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase. Cloning, sequencing, expression, purification, kinetic analysis, and domain mapping. J Biol Chem 271:2225–2233. https://doi.org/10.1074/jbc.271.4.2225

Rose MG, Farrell MP, Schmitz JC (2002) Thymidylate synthase: a critical target for cancer chemotherapy. Clin Colorectal Cancer 1:220–229. https://doi.org/10.3816/CCC.2002.n.003

Saevarsdottir S et al (2011) Predictors of response to methotrexate in early DMARD naive rheumatoid arthritis: results from the initial open-label phase of the SWEFOT trial. Ann Rheum Dis 70:469–475. https://doi.org/10.1136/ard.2010.139212

Selga E, Oleaga C, Ramirez S, de Almagro MC, Noe V, Ciudad CJ (2009) Networking of differentially expressed genes in human cancer cells resistant to methotrexate. Genome Med 1:83. https://doi.org/10.1186/gm83

Sergeant JC et al. (2018) Prediction of primary non-response to methotrexate therapy using demographic, clinical and psychosocial variables: results from the UK Rheumatoid Arthritis Medication Study (RAMS) Arthritis Res Ther 20:147 https://doi.org/10.1186/s13075-018-1645-5

Sharma S et al (2009) Purine biosynthetic pathway genes and methotrexate response in rheumatoid arthritis patients among north Indians.

Pharmacogenet Genomics 19:823–828. https://doi.org/10.1097/fpc.0b013e328331b53e
Shevach EM, Tran DQ, Davidson TS, Andersson J (2008) The critical contribution of TGF-beta to the induction of Foxp3 expression and regulatory T cell function. Eur J Immunol 38:915–917. https://doi.org/10.1002/eji.200738111
Singh JA et al (2016) 2015 American college of rheumatology guideline for the treatment of rheumatoid arthritis. Arthritis Care Res (Hoboken) 68:1–25. https://doi.org/10.1002/acr.22783
Slauenwhite D, McAlpine SM, Hanly JG, Malik A, Haidl ID, Marshall JS, Issekutz TB (2020) Type 2 polarized t cell phenotype is associated with methotrexate non-response in patients with rheumatoid arthritis. Arthritis Rheumatol. https://doi.org/10.1002/art.41223
Smolen JS et al (2020) EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2019 update. Ann Rheum Dis. https://doi.org/10.1136/annrheumdis-2019-216655
Stamp LK, Hazlett J, Highton J, Hessian PA (2013) Expression of methotrexate transporters and metabolizing enzymes in rheumatoid synovial tissue. J Rheumatol 40:1519–1522. https://doi.org/10.3899/jrheum.130066
Stempak JM, Sohn KJ, Chiang EP, Shane B, Kim YI (2005) Cell and stage of transformation-specific effects of folate deficiency on methionine cycle intermediates and DNA methylation in an in vitro model. Carcinogenesis 26:981–990. https://doi.org/10.1093/carcin/bgi037
Sun W et al (2019) Correlations between the polymorphism of +869T/C in TGF-β1 and rheumatoid arthritis. J Musculoskelet Neuronal Interact 19:127–132
Tengstrand B, Ahlmen M, Hafstrom I (2004) The influence of sex on rheumatoid arthritis: a prospective study of onset and outcome after 2 years. J Rheumatol 31:214–222
Tsujimura S, Tanaka Y (2015) Disease control by regulation of P-glycoprotein on lymphocytes in patients with rheumatoid arthritis World. J Exp Med 5:225–231. https://doi.org/10.5493/wjem.v5.i4.225
van der Heijden JW et al (2009) Involvement of breast cancer resistance protein expression on rheumatoid arthritis synovial tissue macrophages in resistance to methotrexate and leflunomide. Arthritis Rheum 60:669–677. https://doi.org/10.1002/art.24354
van Hamburg JP et al (2011) Th17 cells, but not Th1 cells, from patients with early rheumatoid arthritis are potent inducers of matrix metalloproteinases and proinflammatory cytokines upon synovial fibroblast interaction, including autocrine interleukin-17A production. Arthritis Rheum 63:73–83. https://doi.org/10.1002/art.30093
van Vollenhoven RF et al (2009) Addition of infliximab compared with addition of sulfasalazine and hydroxychloroquine to methotrexate in patients with early rheumatoid arthritis (Swefot trial): 1-year results of a randomised trial. Lancet 374:459–466. https://doi.org/10.1016/s0140-6736(09)60944-2
Varani K, Massara A, Vincenzi F, Tosi A, Padovan M, Trotta F, Borea PA (2009) Normalization of A2A and A3 adenosine receptor upregulation in rheumatoid arthritis patients by treatment with anti-tumor necrosis factor alpha but not methotrexate. Arthritis Rheum 60:2880–2891. https://doi.org/10.1002/art.24794
Varani K, Padovan M, Vincenzi F, Targa M, Trotta F, Govoni M, Borea PA (2011) A2A and A3 adenosine receptor expression in rheumatoid arthritis: upregulation, inverse correlation with disease activity score and suppression of inflammatory cytokine and metalloproteinase release. Arthritis Res Ther 13:R197. https://doi.org/10.1186/ar3527
Verburg RJ et al (2005) Outcome of intensive immunosuppression and autologous stem cell transplantation in patients with severe rheumatoid arthritis is associated with the composition of synovial T cell infiltration. Ann Rheum Dis 64:1397–1405. https://doi.org/10.1136/ard.2004.033332
Vilca I et al (2010) Predictors of poor response to methotrexate in polyarticular-course juvenile idiopathic arthritis: analysis of the PRINTO methotrexate trial. Ann Rheum Dis 69:1479–1483. https://doi.org/10.1136/ard.2009.120840
Visser K et al (2008) Pretreatment serum levels of anti-cyclic citrullinated peptide antibodies are associated with the response to methotrexate in recent-onset arthritis. Ann Rheum Dis 67:1194–1195. https://doi.org/10.1136/ard.2008.088070
Volk EL, Rohde K, Rhee M, McGuire JJ, Doyle LA, Ross DD, Schneider E (2000) Methotrexate cross-resistance in a mitoxantrone-selected multidrug-resistant MCF7 breast cancer cell line is attributable to enhanced energy-dependent drug efflux. Cancer Res 60:3514–3521
Wang YC, Chiang EP (2012) Low-dose methotrexate inhibits methionine S-adenosyltransferase in vitro and in vivo. Mol Med 18:423–432. https://doi.org/10.2119/molmed.2011.00048
Wang HX et al (2018) Dysregulated ICOS(+) proinflammatory and suppressive regulatory T cells in patients with rheumatoid arthritis. Exp Ther Med 16:3728–3734. https://doi.org/10.3892/etm.2018.6657
Wang J, Mao N, Xie X, Li S, Chen WJ (2019) High expression of multidrug resistance gene-1 can aggravate resistance to methotrexate in rheumatoid arthritis patients. Zhongguo Yi Xue Ke Xue Yuan Xue Bao. Acta Acad Med Sin 41:595–600. https://doi.org/10.3881/j.issn.1000-503x.10927
Wehrens EJ et al (2011) Functional human regulatory T cells fail to control autoimmune inflammation due to PKB/c-akt hyperactivation in effector cells. Blood 118:3538–3548. https://doi.org/10.1182/blood-2010-12-328187
Wei W (2016) Soft regulation of inflammatory immune response. Chin Pharmacol Bull 32:297–303
Wojtuszkiewicz A et al (2015) Methotrexate resistance in relation to treatment outcome in childhood acute lymphoblastic leukemia. J Hematol Oncol 8:61. https://doi.org/10.1186/s13045-015-0158-9
Wolf J, Stranzl T, Filipits M, Pohl G, Pirker R, Leeb B, Smolen JS (2005) Expression of resistance markers to methotrexate predicts clinical improvement in patients with rheumatoid arthritis. Ann Rheum Dis 64:564–568. https://doi.org/10.1136/ard.2003.014985
Wong LH, Flibotte S, Sinha S, Chiang J, Giaever G, Nislow C (2017) Genome-wide screen reveals sec21 mutants of saccharomyces cerevisiae are methotrexate-resistant. G3 (Bethesda) 7:1251–1257. https://doi.org/10.1534/g3.116.038117
Wu YJ, Wang C, Wei W (2018) The effects of DMARDs on the expression and function of P-gp, MRPs, BCRP in the treatment of autoimmune diseases. Biomed Pharmacother 105:870–878. https://doi.org/10.1016/j.biopha.2018.06.015
Yao X et al (2017) Over-expression of mdr1/P-gp is associated with methotrexate resistance in patients with rheumatoid arthritis. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi. Chin J Cell Mol Immunol 33:815–819
Youssef P, Roth J, Frosch M, Costello P, Fitzgerald O, Sorg C, Bresnihan B (1999) Expression of myeloid related proteins (MRP) 8 and 14 and the MRP8/14 heterodimer in rheumatoid arthritis synovial membrane. J Rheumatol 26:2523–2528
Yu J, Zhou P, Asenso J, Yang XD, Wang C, Wei W (2016) Advances in plant-based inhibitors of P-glycoprotein. J Enzyme Inhib Med Chem 31:867–881. https://doi.org/10.3109/14756366.2016.1149476
Yu MB, Firek A, Langridge WHR (2018) Predicting methotrexate resistance in rheumatoid arthritis patients. Inflammopharmacology 26:699–708. https://doi.org/10.1007/s10787-018-0459-z