Chapter

Immunomodulatory Effect of Methotrexate Abruptly Controls Keratinocyte Activation in Psoriasis

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Abstract

In psoriatic skin, epidermal keratinocytes (KCs) undergo deregulated inflammatory response that leads to prolonged expression of inflammatory mediators as well as abnormal keratins. Due to immune and genetic factors, KCs get activated and cell balance gets disturbed. This activation is mainly due to deregulated inflammatory response. A vicious cycle of KC-immune response called KC activation cycle leads to psoriasis. In psoriatic skin, epidermal KCs undergo deregulated inflammatory response that leads to prolonged expression of inflammatory mediators as well as abnormal keratins. Methotrexate (MTX) an immunosuppressive agent has been used as a standard drug to treat severe psoriasis. Acanthosis and abnormal terminal differentiation was mainly due to the mutation in epidermal keratins. In turn, disease severity and relapsing of psoriasis are mainly due to the mutation of hyperproliferative keratins. These novel keratin mutations in psoriatic epidermis might be one of the causative factors for psoriasis. MTX strongly regulates the KC activation cycle by deregulated inflammatory markers and maintains normal keratin phenotype on hyperproliferating KC, thereby controlling acanthosis in psoriasis patients.

Keywords: psoriasis, methotrexate, keratins, inflammatory markers, keratinocyte

1. Introduction

Psoriasis is a chronic inflammatory skin disease that mainly characterized by acanthosis, abnormal differentiation and infiltration of leukocytes from the dermis. The factors that causing this disease are genetic, environmental and inflammatory mediators [1]. In normal skin, the transformation of basal keratinocytes (KCs) to anucleate corneocytes process will takes within 50 days. Whereas, in psoriatic skin the epidermal cell cycle is rapid and the transformation occurs within 5 days. Thereby, the stratum corneum contains fully unmatured keratinized cells which build up abnormally and forms scales like structure. Due to this, epidermis of psoriatic lesions will become thicker and also blood vessels in the papillary layer of the dermis get dilated along with effusion of inflammatory cells, such as neutrophils, infiltrate the epidermis [2]. About 80% of the epidermal skin constitutes KCs. KC play a major role

in this chronic inflammatory disease. It play a special role in sensing epidermal barrier and regulating immune homeostasis [1].

1.1 Hyperproliferation of epidermis in psoriasis

Until the late 1970s, the hypothesis of psoriasis arising from abnormalities in KCs was favored, and the abnormal proliferation was treated with antiproliferative agents. Since then, the participation of KC in the pathogenesis of psoriasis has certainly been overlooked. A publication by Zenz et al. [3] has highlighted again the role of KC in the pathomechanisms leading to psoriatic lesions. Their findings favor the view that psoriasis could also be regarded as a primary KC disorder amplified by the immune system [4]. While the debate will continue among skin biologists on the different theories, it is unquestionable that KC are potential initiators of inflammation, producing a number of cytokines, adhesion molecules and growth factors.

The growth of KC is regulated by a delicate balance between molecules that control cell survival and cell death. Thus, the thickness of human epidermis remains relatively constant throughout life. This regulation is disturbed in psoriasis that leads to KC hyperproliferation with the net result of an increase in the volume of cell mass [5]. The epidermal cell cycle of hyperproliferating psoriatic KC occurs within 5 days. Effusion of growth factors and inflammatory mediators from different skin cells, are believed to regulate the epidermal hyperproliferation in psoriasis (**Figure 1**) [6].

When skin cells exposed to any external factors such as environmental, chemical and internal factor like genetic, psychological and physical stress will probably activate immune cells within the KCs, which create KCs to hyperproliferate and also altered differentiation. Thus, the establishment of mutual KC-immunocyte stimulation (KC activation cycle) will leads to psoriasis [7, 8]. The hyperproliferation in psoriasis seemed to result from an increase in the number of transit amplifying cells, following depletion of the stem cell compartment [9]. As a whole, these changes suggest that intermediate filament (IF) keratin pair provides specific functional requirements to maintain epidermal KCs. KCs stability and integrity are mainly depend on keratin proteins [10, 11]. Keratins are the main structural cytoskeletal protein, an IF in all epithelia [12]. In normal skin, basal KCs express K5 and Keratin 14 (K14) keratins which helps in proliferation, whereas suprabasal cells express K1 and Keratin 10 (K10) keratins which supports differentiation process [13, 14]. Subsequently, any defects in these keratins can lead to cell fragility and are linked to a wide array of genodermatoses and cancers [15, 16].

Since genome-wide association studies (GWAS), connecting the psoriasis to the late cornified envelope gene cluster has specified that epidermal abnormalities along with hyperproliferative keratin pattern plays a major role in the pathogenesis of psoriasis [17, 18]. Studies have shown that dysfunction or mutations of keratin proteins are associated with a remarkable variety of skin disorders, such as skin blistering, inflammatory disorders and skin tumors [19]. The main aim of psoriasis treatments is to stop skin cells from growing so quickly and to remove scales. Methotrexate (MTX) is considered as the gold standard therapy for moderate to severe psoriasis [20–23]. Mostly, MTX exerts various immunomodulatory effects on T cell and also control KC growth [24]. In psoriasis, MTX was found to decreases the markers involved in hyperproliferation [25].

In this chapter we will first describe KC hyperproliferation then how keratins are expressed and regulated in psoriasis, then we will describe how MTX exert its action on controlling psoriasis through its immunomodulatory effect on Keratins and KC activation.

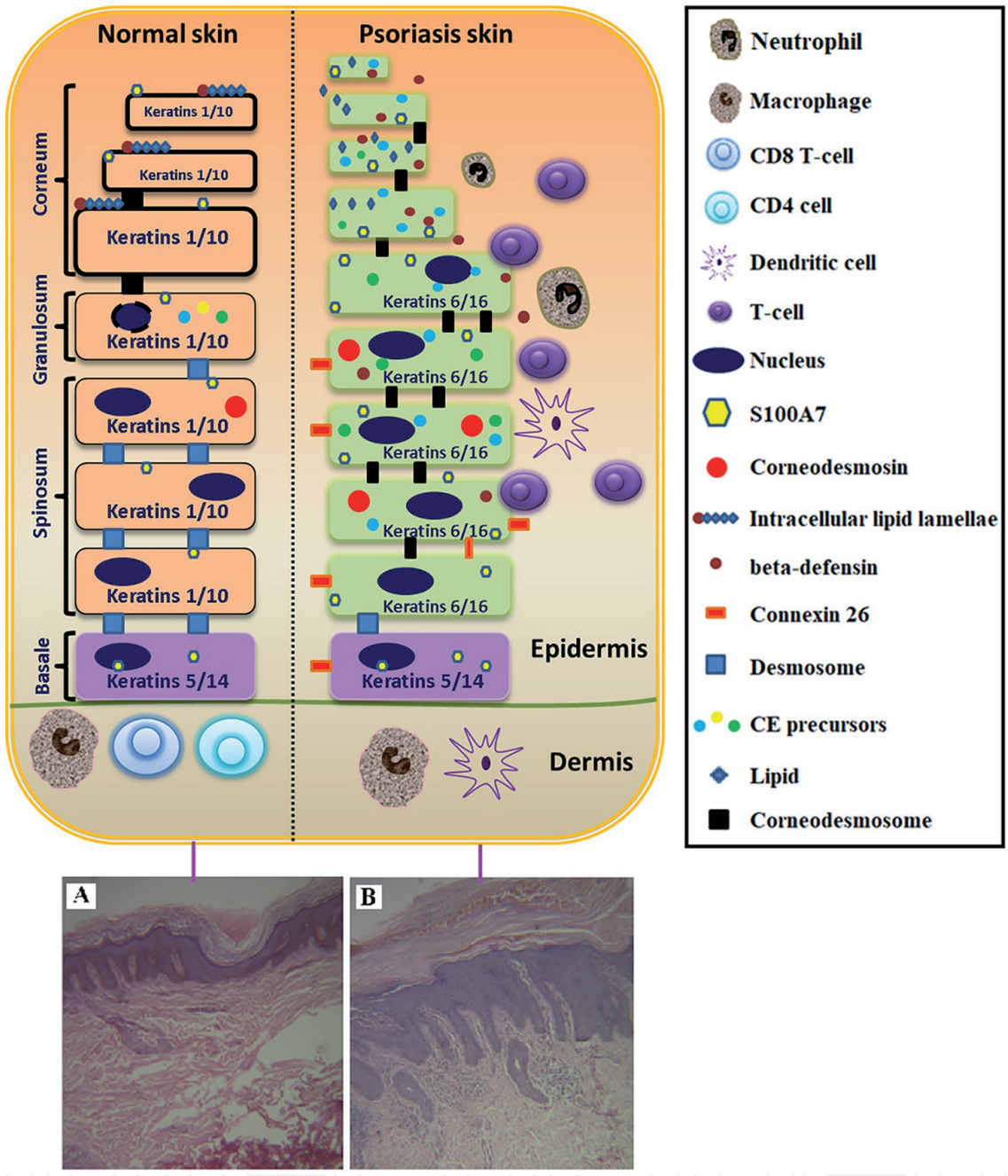


Figure 1.
Illustration of the epidermal layers of the normal skin and psoriatic skin including scaliness, hyperkeratosis, and neutrophil accumulation in the stratum corneum. A indicates immunohistology of normal skin, B indicates immunohistology of psoriatic skin.

2. Keratins involved in psoriasis

The hyperproliferation seemed to result from an increase in the number of transit amplifying cells, following depletion of the stem cell compartment [9, 26]. The differentiation state of epidermal KCs is reflected by the intricate expression pattern of keratins [27, 28]. Keratins are members of the large IF gene family [29] in all epithelia including the epidermis. Basal KCs express keratins (K) K5 and K14, which helps to maintain epidermal shape are replaced by differentiation keratin K1 and K10 [27], whereas in activated KCs, keratins K6, K16, and Keratin 17 (K17), which are distinct from the keratins in the healthy epidermis, are expressed [30]. Therefore, keratins K6,

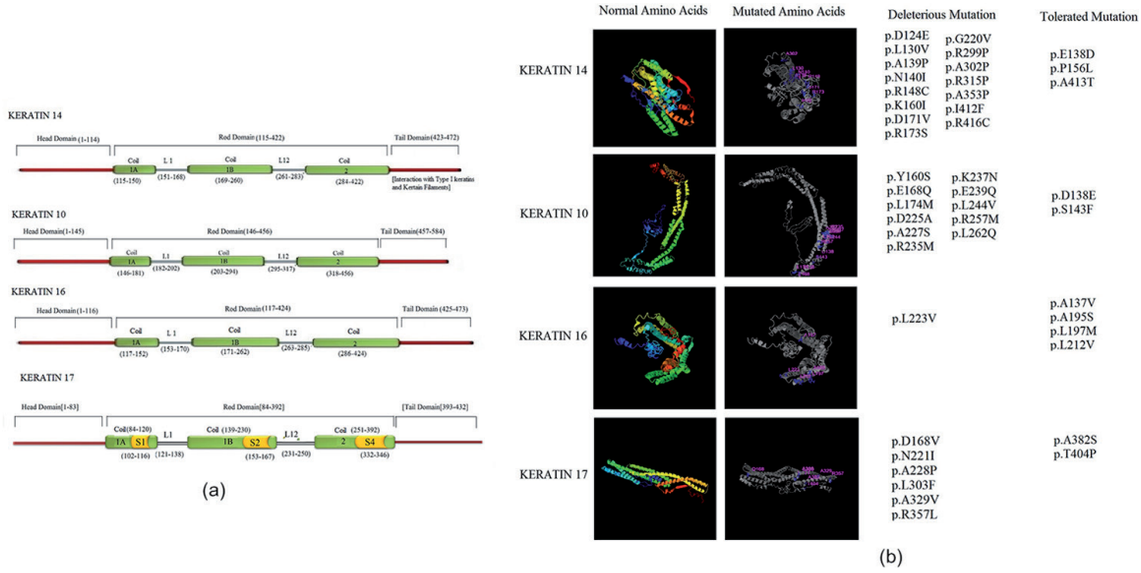


Figure 2.
Protein structure of epidermal and hyperproliferative keratins protein. (a) Schematic representation of secondary structure of all four keratins with their domain and sub domain. Whereas, S = peptide epitope; L = linker. (b) Region specific of 33 deleterious mutation localization. The left sides of the figures are the 3D structure of all four keratins CDS region. The right sides of the figures are the 3D structures of domains with mutated residues. The mutated residues are numbered according to their position on regions. The position of mutated amino acids (aa) on all four keratins and on CDS regions are provided [31].

K16, and K17 are usually referred to as activation- and hyperproliferation-associated keratins. There are predicted deleterious mutations were highly located in α-helical rod domain of keratins which forms coiled structure to these keratins, are important to maintain the structural integrity of the skin. Thus the genetic defects of basal keratin K14 and differentiation keratin K10 in skin might leads to express abnormal keratin pair which causes thickening of epidermis (acanthosis) in psoriasis (**Figure 2**).

2.1 Keratin 14

Keratin 5 and K14 pair are considered as a biochemical marker of mitotically active basal layers. This pairs are supports to maintain epidermal integrity as well as protects skin from mechanical stress. Interestingly, the K5/K14 pair is expressed in the basal layer of the epidermis, which contains epidermal stem cells and transient amplifying (TA) cells [32]. Many studies have showed that the level of K14 was considerably higher in lesional skin than in normal epidermis [33–35]. p53 activation promotes the expression of p21 and the repression of K14 during epidermal differentiation [36], whereas in psoriasis, there is downregulation of p53 [37] and also decreased Notch 1 expression [38] causes increase in K14 expression in psoriasis [39]. p75NTR is nearly absent in psoriatic KCs that are reportedly resistant to apoptosis, leads to increase TA cell turnover in psoriasis which leads to increase in K14 expression in psoriasis (**Figure 3**) [40].

2.2 Keratin 10

The epidermis is a stratified epithelium that regenerates permanently from the basal layer. K10 pair with K1 is considered as a major differentiation keratin [29]. Normally,the basal layer contains K14, K15, and K5, whereas suprabasal, postmitotic

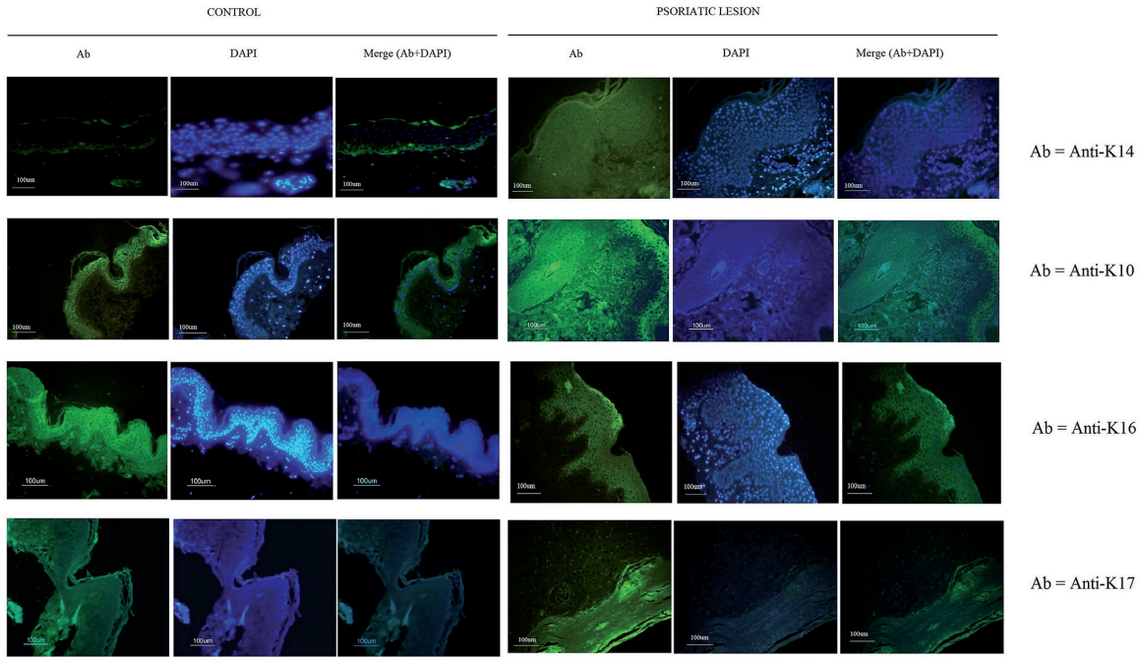


Figure 3.
Immunofluorescence analysis of keratins in control and lesional psoriatic skin. Immunofluorescence analysis of epidermal and hyperproliferative type I keratins in frozen skin sections from patients with psoriatic skin (×20 magnification, respectively), and nuclei were visualized with 4′-6-diamidino-2-phenylindole (DAPI). Bar = 100 μm. Ab = antibody [31].

cells switch to the expression of K1 and K10 [41, 42] is also involved in the control of cell proliferation [43]. Many studies have shown a downregulation of K1 and K10 [33–35] in psoriatic epidermis. This may be due to downregulation in E-cadherin expression [43], and increase in IL-22 [45–48] levels in psoriasis. Also c-fos protein in AP-1 transcription factor highly regulates a number of genes that are involved in KC differentiation were found to be reduced in psoriasis [49–52].

In psoriasis, defects in K10 expression leads to hyperproliferation of KCs by decreasing its inhibitory action on Rb phosphorylation, which leads to increased Cyclin D and E expression and also increased Phosho-Akt levels in psoriasis (**Figure 3**) [52].

2.3 Keratin 16

Keratin 16 expressions, reduces the fraction of cells in G1 while increasing that in S phase [52]. Many authors also shown an upregulation of K6 and K16 [33, 35, 54, 55] in psoriatic epidermis. Decrease expression of keratin 10 gene leads to hyperproliferation of basal cells, alterations in epidermal cell cycle by inducing cmyc, cyclin D1, 14-3-3σ, keratin 6 and keratin 16 [56]. Multiple transcription factors activated by the extracellular signals revealed the specific signals transduction mechanisms that respond to the corresponding growth factors and cytokines. For example, interleukin-1 (IL-1), present in healthy epidermis in inactive form [57], when released autocrinely, activates NFκB and C/EBPβ, thus initiating KC activation. Among the characteristics of activated KCs is the production of tumor necrosis factor-α (TNF-α) [58], which maintains activated NFκB and C/EBPβ. Activated KCs produce ligands of the EGF receptor that cause activation of AP1, such as transforming growth factor-α (TGF-α), amphiregulin and HB-EGF. Interestingly, all these cytokines and

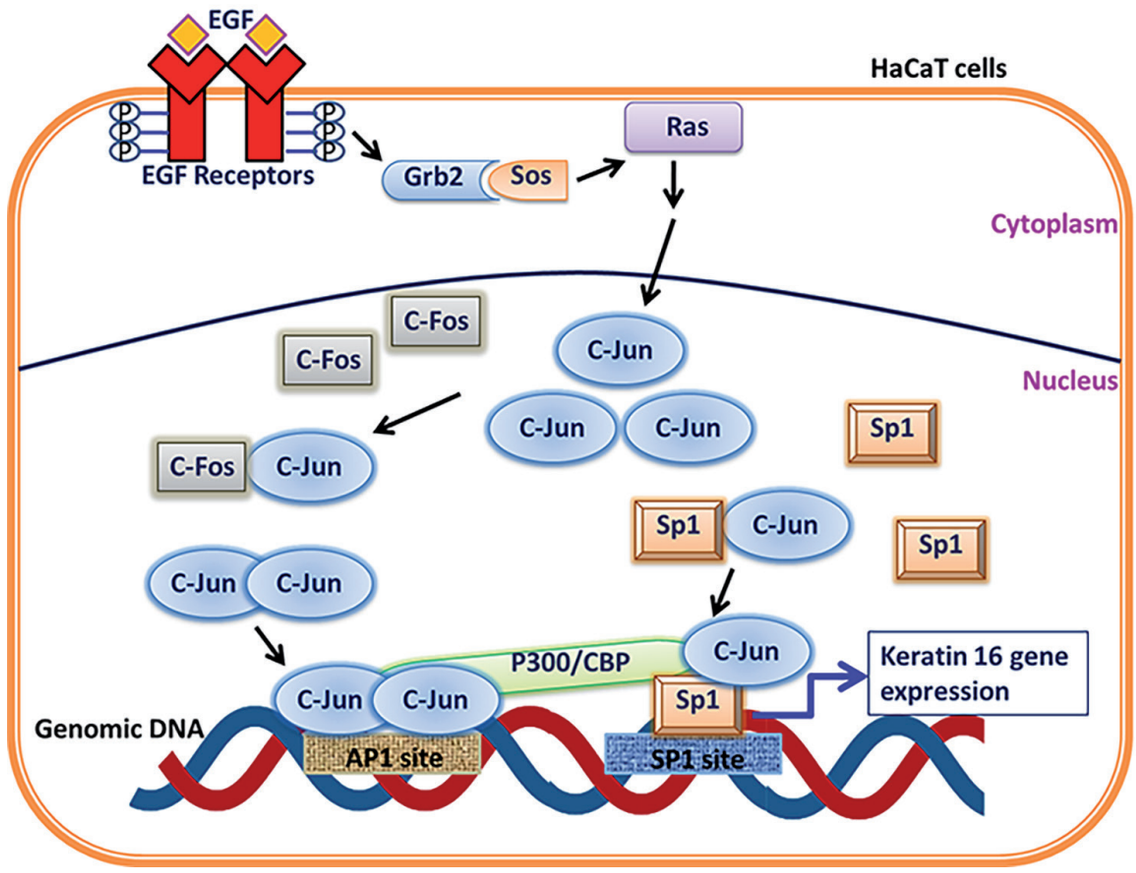


Figure 4.
Transcriptional regulation of the human keratin 16 gene in HaCaT cells.

growth factors IL-1, TNF-α, TGF-α and EGF, induce expression of the K16 genes, albeit through separate pathways [54, 59–61]. Study has shown that biosynthesis of AP1 protein (c-Jun and c-Fos) through Ras activation are actively participate in the transcriptional regulation for EGF which induced keratin 16 gene expression in KCs [62], this implies that EGF induces Keratin 16 expression in psoriasis (**Figure 4**).

2.4 Keratin 17

Keratin 17 (K17) is a type I intermediate filament mainly expressed in the basal cells of epithelial in hair follicles. As a complex cytoskeletal protein, K17 regulates a numereous biological processes in epithelial layer, including cell proliferation and growth, skin inflammation and hair follicle cycling. Abnormal expression of K17 is found in various diseases ranging from psoriasis to malignancies such as breast, cervical, oral squamous and gastric carcinomas [63, 64].

Many studies have showed the aberrant expression of K17 in psoriatic epidermis [35, 65, 66]. This aberrant expression is mainly due to interferon-γ (IFN-γ) produced by activated T cell [67]. Various inflammatory mediators like IFN-γ, IL-6, IL-17 A, IL-22 derived from T cells induced transcription of keratin K17 through STAT3- and ERK1/2-dependent mechanisms [64, 68, 69]. Thus, all these cytokines are involved in the induction of K17/T cell/cytokine autoimmune loop and play an important role in the progression of psoriasis [68].

3. Keratinocyte activation cycle (KAC)

Basal KC have two alternative pathways to end up. In normal skin, KCs undergo proliferation in basal layer and differentiation in both spinosus, granular layer, finally it end up as anucleate corneocytes in cornified layers. During all these process, various factors like calcium, retinoic acid, vitamin D3 and protein kinase C (PKC) activators are required to induce proliferation and differentiation in epidermis [70–74]. For proliferation and differentiation, Epidermal KCs required a pair of kertain proteins, for instance, in normal skin Keratin pair K5/K14 expressed in basal layer and K1/K10 are expressed in spinosus layer [75].

However, in pathological conditions like epidermal injury, cancer and psoriasis an alternative KCs pathway is get activated by inflammatory mediators. This alternative pathway disturbed normal proliferation and migrating phenotype in KCs [76, 77]. This alternative pathway is considered as a unusual cycle formed by the interaction of KCs-immunocytes, which is precisely elucidated as KC activation cycle [78]. This activation cycle begins with leakage of interleukin-1 beta (IL-1 β) from KC after any injury or disease condition. This inflammatory mediator initiate activation of KCs by changing the keratin pattern from K5/14 to K6/16 and firmly maintained by TNF-α and TGF-α [60, 61, 75, 79]. After any treatment or lesional healing stage, IFN-γ was released by KCs which act as a signal for healing process and also induces the expression of K17 [69, 80]. To normalize the healed skin, a transforming growth factor-β (TGF-β) synthesized from dermal fibroblasts were found to aid KCs phenotype by producing K5/14 keratin in basal layer (**Figure 5**) [81].

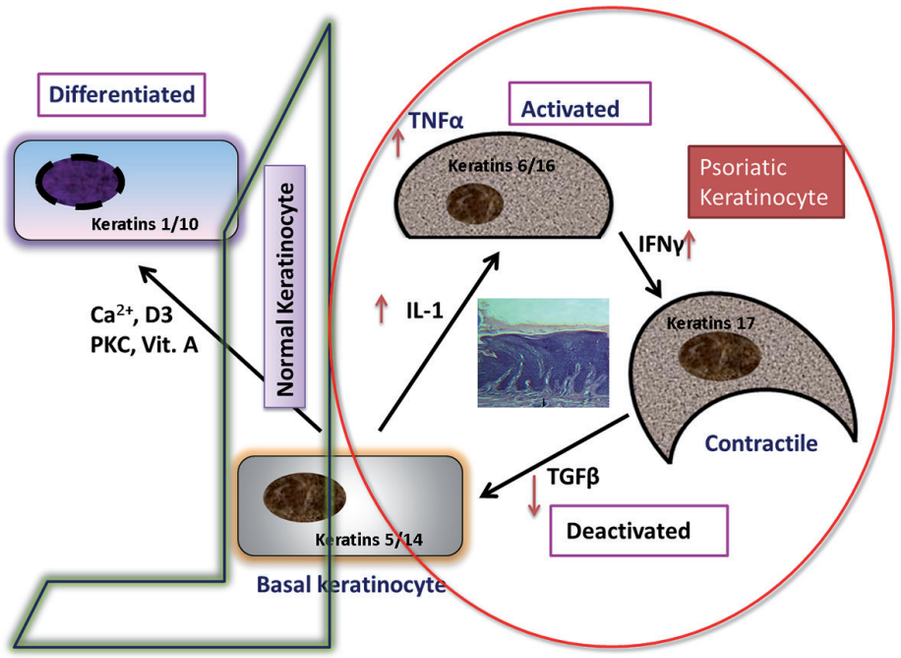


Figure 5.
The KC activation cycle. In normal KC, Basal layer produces K5 and K14 and differentiate to K1 and K10 with the help of ca2+, D3. Whereas in psoriatic KCs, K5 and K14 produce K6 and K16 by IL-1. TNF-α help to maintains activated KCs. Then, IFN-γ prompts K17 expression which stimulates KCs contractility. TGF-γ,a de-activating signal is not produced during psoriass, thereby hyperproliferation of epidermis in psoriasis. Symbol ↑ indicates increase in psoriasis; symbol ↓ indicates decrease in psoriasis.

3.1 Initiator of activation

The most common initiator of KC activation is IL-1. In pathological conditions like psoriasis, KC process and release IL-1, allowing the surrounding cells to perceive it [82–84]. The released IL-1 serves as a paracrine signal to dermal endothelial cells to become activated, express selectins, and slow down the circulating lymphocytes [85, 86]. IL-1 also serves as a chemoattractant for lymphocytes, causing them to extravasate and migrate to the site of lesion [87]. Furthermore, IL-1 is an activator of dermal fibroblasts, enhancing their migration, proliferation, and production of dermal extracellular matrix components [88]. IL-1 is also an autocrine signal that activates KC. IL-1 causes them to proliferate, become migratory, and express an activation-specific set of genes [76]. Thus, IL-1 initiates KC activation not only by triggering additional signaling events, but also by inducing directly the synthesis of K6/K16 in epidermal KC, and thus changing the composition of their cytoskeleton.

3.2 Maintenance of activation

TNF-α induced by IL-1 can maintain KCs in an activated state [75]. In psoriasis, a wide variety of cells produce TNF-α, primarily macrophages and monocytes but also epithelial cells including KCs [89]. TNF-α activates immune responses by inducing production of additional signaling molecules, cytokines, growth factors, their receptors, and adhesion proteins (e.g., amphiregulin, TGF-α, IL-1 α, IL-1 receptor antagonist, epidermal growth factor receptor (EGFR), and intercellular adhesion molecule (ICAM-1) [90]. In response to the activation of the EGFR, KCs proliferate, degrade components of the extracellular matrix, and become migratory [91].

3.3 The activated phenotype

Once activated, KCs synthesize additional signaling growth factors and cytokines including TGF-α IL-3, IL-6, IL-8, G-CSF, GM-CSF, and M-CSF [91, 92]. These signaling molecules produced by KCs act as paracrine signal to white blood cells, lymphocytes, fibroblasts, and endothelial cells in dermis. Apart from paracrine, it also acts as an autocrine signal for KCs in epidermis. Numerous cell surface proteins and integrins are also found to act as secondary moiety to activate epidermal KCs [93, 94].

3.4 The contractile keratinocyte

Psoriasis is associated with high levels of IFN-γ in epidermis [91]. IFN-γ strongly and specifically induced the promoter of the K17 (abnormal marker) gene. K17 is exceptional because it is not found in healthy interfollicular epidermis, but it is expressed in certain pathologic states, psoriasis [28]. The function of K17 in epidermis therefore may be to promote or allow KC contractility and/or frequent changes in shape [95]. Indeed, expression of K17 has been used to evaluate the course of treatment of psoriatic patients [63]. Due to failure, to resolve the deregulated inflammatory response in psoriasis leads to the persistent activation of KCs, which is characterized by prolonged K17 expression [13].

3.5 Back to normal basal phenotype

To revert to the basal cell phenotype, KCs need a signal. This signal comes from the dermal fibroblasts in the form of TGF-β. Cell kinetics study by Van Ruissen et al.

[96] clearly indicate that the TGF-β donot control KCs normal proliferation, but it controls the abnormal proliferation of KCs (antihyperproliferative). Whereas in psoriasis, the expression of TGF-β is low [97]. So the activation cycle is not reverted to normal basal phenotype, therefore hyperproliferation of KC takes place in psoriasis.

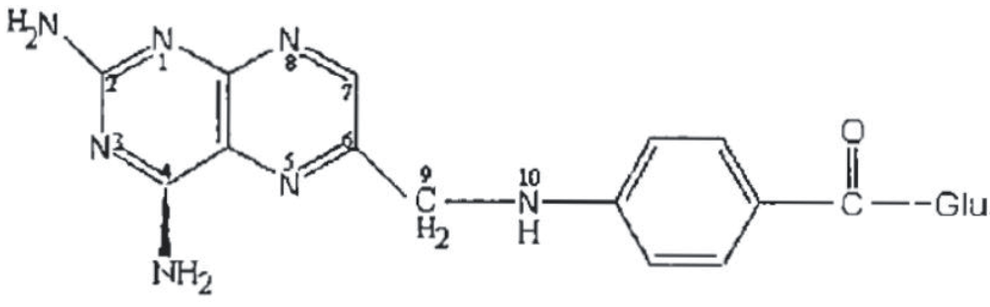
4. Methotrexate

MTX is the most commonly used systemic agent for psoriasis and, because it has been available for 35 years, most dermatologists are comfortable with its use. It is considered as an longstanding effective oral medication for the treatment of various types of psoriasis [98, 99]. Generally psoriasis treatment is mainly depends on the PASI score. For mild psoriasis patients, doctors prescribed topical therapy and phototherapy. For moderate to severe disease who have at least 5% of their skin covered with psoriasis are treated with oral medications like MTX, cyclosporine. [100]. Folate supplementation along with MTX reduces the incidence of megaloblastic anemia, hepatotoxicity, and gastrointestinal intolerance [101].

4.1 History

Anti-metabolites mimic substances required for normal biochemical reactions and thus interfere with normal functions of the cell, including cell division. They may masquerade as purines (e.g., azathioprine), pyrimidines (e.g., 5-flourouracil) and folic acid analogs essential for purine and pyrimidine synthesis, (e.g., MTX). Methotrexate, formerly known as aminopterin, has been widely used in the treatment of cancer and autoimmune diseases [102]. It was first developed in the 1940s when scientists were investigating the effects of folic acid on cancer, particularly childhood leukemia [103]. MTX is an analog of folic acid that inhibits cellular proliferation inducing folate coenzyme deficiencies [104]. When MTX was incidentally noted to improve psoriatic lesions in the 1960s it became clear that it possessed anti-inflammatory properties in addition to its antiproliferative effects [105–107]. Anti-inflammatory actions of MTX is accomplished by inhibiting dihydrofolate reductase which eventually diminishes the de novo synthesis of purines and pyrimidines and increases the catabolism of AMP and adenosine to IMP and inosine. Increased catabolism leads to accumulation of adenosine which confers anti-inflammatory effects of MTX [108].

4.2 Chemical structure of methotrexate



(2S)-2-[[4-[[2,4-bis (azanyl)pteridin-6-yl]methyl-methylamino]phenyl]carbonylamino] pentanedioic acid.

5. Clinical efficacy of methotrexate on psoriasis

Even though MTX has been used extensively for the treatment of psoriasis, so far its efficacy has not been supported by any clinical datas. A non-randomized controlled trial study done by our research team showed PASI 75 was achieved in 75% of psoriasis patients after 7.5 mg of MTX orally per week for a period of 12 weeks. PASI 75 is defined as a reduction from baseline PASI score of >75%. PASI 75 is used as the benchmark of primary end points in assessing therapies for psoriasis. Patients reaching PASI 75 represent very meaningful changes in psoriasis severity [109–112]. A randomized controlled clinical trial using 15 mg of MTX for 16 weeks in moderate to severe plaque has showed 60% of patients achieved a PASI-75 response (no significant difference, $P = 0.29$), [113, 114]. For safety consideration, MTX has found to be administered along with 5 mg of folic acid to minimize the serious toxicity include hematological disorder, hepatotoxicity and gastrointestinal toxicity, MTX has also affect lymphocytes and also cause autoimmune reaction at extreme usage [115–117]. But, several studies has shown that 5–10 mg of MTX cause less side-effect on liver of

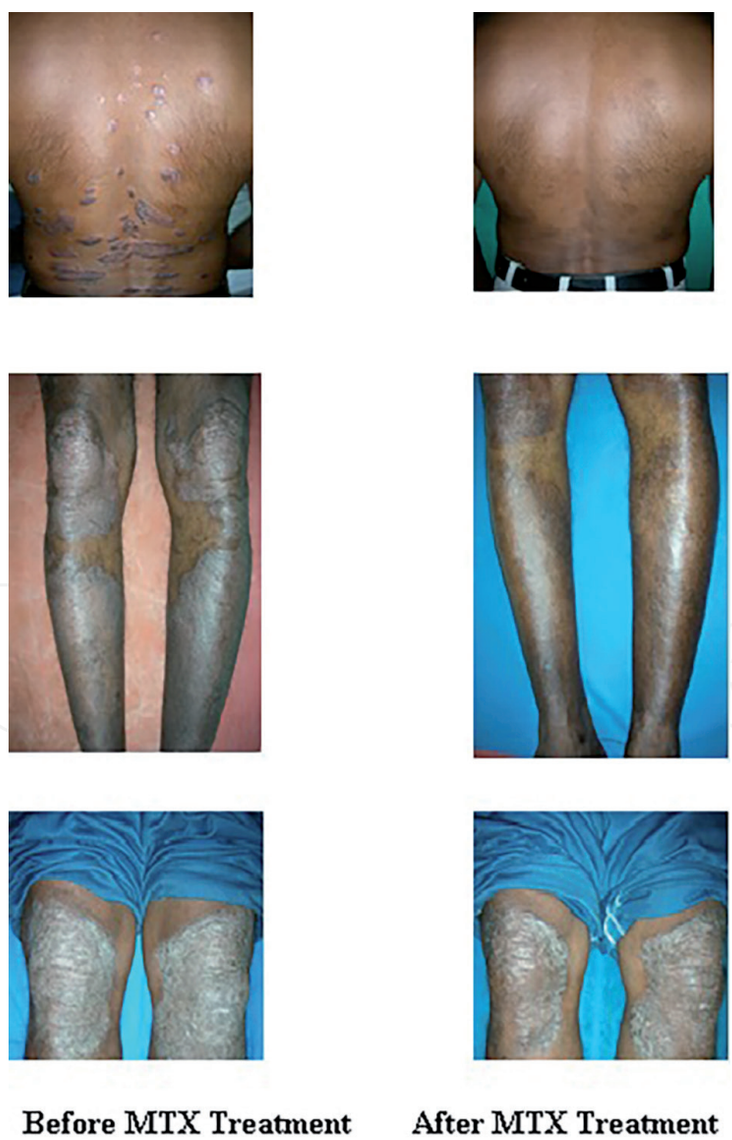


Figure 6.
Clinical efficacy of MTX on psoriatic patients.

psoriasis patients [118–121]. However more than 15 mg will cause severe side effects in psoriasis patients [121]. Clinical improvement of psoriatic patients before and after treatment of 7.5 mg of MTX per week for 12 weeks were depicted in (**Figure 6**).

Overall, the clinical data from our research study and also from other clinical trial has strongly supports that using low dose of MTX is well and safe for the treatment of moderate to severe psoriasis patients, and also frequent expert supervision along with laboratory monitoring is necessary.

6. Metabolic activity of methoxerate on psorisis

MTX mechanisms of action are likely to account for its antiproliferative and immunosuppressive effects [122, 123]. The key feature of psoriasis is KC hyperproliferation. MTX was found to induce maturation and inhibition of KC proliferation through its metabolic action of kerationocyte. The putative effects of MTX are listed below:

1. Reduction of cell proliferation

2. Increase of apoptosis of T cells

3. Increase of endogenous adenosine release

4. Alteration of expression of cellular adhesion molecules and

5. Influence on production of cytokines, humoral responses, and bone formation.

6.1 Effect of methotrexate on T cells

Evidence supports that activated T cells are key players in the immunopathogenesis of psoriasis. The following components involving T cells are considered crucial in the pathogenesis of psoriasis. Activated endothelium of psoriatic skin has shown adhesion molecules like E-selectin, CLA, ICAM-1 and ICAM-3, which promoted the activation of KCs and T-cells in psoriasis. Study by Sigmundsdottir et al. showed that treatment of 5–25 mg of MTX in 16 moderate to severe psoriasis patients has showed decreased E-selectin and CLA expression in psoriatic skin. Thus, the downregulation of peripheral Tcell-adhesion interaction by MTX in psoriasis patients implies its therapeutic action on psoriatic skin lesion [124]. Invitro flowcytometric and immunohistochemistry studies have shown that peripheral T cells were found to show less interaction with CLA and ICAM-1 after MTX administration 10^{-9} M to 10^{-5} M for 5 days. Follow-up experiments revealed that MTX suppression of CLA expression could be reversed by folinic acid (leucovorin) supplementation [123].

MTX has also target T cell by inducing cytolysis. Some studies has shown that MTX induce Tcell apoptosis in more sensitive manner [125–127]. MTX may induce cell death via free radical oxygen species. Phillips et al. [126] inhibited MTX induced T-cell death with the addition of the antioxidant glutathione and its precursor, Nacetylcysteine. Accumulating evidence suggests that MTX alters T-cell production of several cytokines, including IL-1, IL-2, IL-4, IL-8, INF-γ and TNF-α [128–131]. As a key element in psoriasis pathogenesis, the cytokine TNF-α was found at higher levels in psoriasis plaques and the synovial fluid of patients with psoriatic arthritis [129,

132, 133]. Associations between MTX and TNF-α levels have been observed since the 1990s. Studies by Seitz et al. [132], Neurath et al. [134] and Hildner et al. [135] found that MTX had reduced TNF-α production in the peripheral blood mononuclear cells (PBMCs) of psoriasis patients.

Action of MTX on reducing serum and synovial TNF-α has been widely established in both Psoriasis and rheumatoid arthritis patients showed its immunomodulatory effects [136–138]. Based on the stage and route of T-cell activation, it has been evident that MTX inhibits T-cell TNF-α production [129, 130, 135]. These all findings suggest that MTX can diminish TNF-α produced by activated T cells showed its immunomodulatory action.

6.2 Effects of methotrexate on endothelial cells

T-cell migration from the intravascular space into the dermis is a crucial step in the pathogenesis of psoriasis, and this process is dependent on interactions between endothelial cells and T cells. Endothelial expression of appropriate adhesion ligands such as E-selectin and ICAM-1, are necessary for successful T-cell adhesion and migration [124, 139]. Studies have shown that MTX treatment firmly decreased the CLA, ICAM-1 and E-selectin expression in the endothelial cells [24].
Histologically, hypervascularity is noted in psoriatic skin, which contributes to the grossly observed erythema. When used at the high dosages necessary for chemotherapy, MTX is capable of inhibiting angiogenesis. MTX exerted its therapeutic effects in psoriasis by inhibiting angiogenesis.In 2003 Yamasaki et al. [140] found that MTX had an inhibitory effect on endothelial cell growth. Two years later, in 2005, Yazici et al. [25] employed immunohistochemistry on lesional skin biopsies to study the effects of MTX on angiogenesis, reporting a statistically significant decrease in the endothelial marker CD31 after treatment with MTX.

Dendritic cells (DCs) are considered as a key player in the pathogenesis of psoriasis. Interplay between DCs, T-cells and cytokine are main and complex in psoriasis. DCs are well recognized has antigen-presenting cells (APC) in the skin. Any modulation in APC interaction with other cells may significantly influence development of psoriatic lesions. Many studies has showed that MTX showed its immunomodulatory effect by suppressing APCs activity [141, 142]. Recently, the use of T-cell targeted therapy confirmed the critical role of lymphocytes [143]. On the other hand, the clinical phenotype observed in psoriasis is mostly accounted for by several alterations in epidermal KCs [144].

7. Action of methotrexate on activated KC-immunocyte cycle in psoriasis

The epidermis is a multilayered epithelium consisting mainly of proliferating and differentiated, postmitotic KCs [145]. The latter derive from transit amplifying cells originating from stem cells that represent a restricted number of basal KCs [146, 147]. The proliferation and differentiation of epidermal KCs is regulated by a multitude of signaling cascades and transcription factors including Wnt/b-catenin [148], growth factors of the EGF and FGF family [149], TGF-β, members of the NFkB family [150], and c-Myc [151].

7.1 Activation and deactivation signals of KAC

In psoriasis, KCs undergo activation pathway. This activation process is governed by growth factors and cytokines, such IL-1, TNF-α, IFN-γ and TGF-β [78].

7.1.1 Interleukin-1

IL-1 is likely to be an important mediator in the initiation and maintenance of psoriatic plaques and may represent an attractive therapeutic target [152].

7.1.1.1 Interleukin-1 alpha

Our studies in IL-l α levels and its action in psoriasis had clearly showed that IL-l α level is reduced in psoriatic skin as well as in plasma [153], which was further supported by several studies [154–157]. Mechanism behind this reduction is mainly depend on the upstream level of nerve growth factor, a IL-1 α down-regulator [158, 159] in psoriasis.

The second messenger cyclic adenosine monophosphate (cAMP) has been regarded as a regulator for cell growth and proliferation [160]. The action of IL-l α is mainly depends on its phosphorylation by cyclic cAMP-dependent protein kinase. Thus, phosphorylation converts it into active form and enhanced its susceptibility to tryptic digestion, which may allow its release into the extracellular milieu [161]. Reduced cAMP levels has been reported in psoriasis [162]. This also could lead to reduced IL-l α levels in lesional skin biopsies.

Studies showed that MTX significantly increased IL-l α levels in plasma and skin biopsies of psoriasis patients. This may due to increase in the levels of cAMP by MTX through adenosine release [153, 163] and that led to an increase in IL-1 α level.

7.1.1.2 Interleukin-1 beta and caspase-1

Many studies have reported the importance of IL-1 β and caspase-1 (IL-1 β converting enzyme) in pathogenesis of psoriasis [153, 156, 157, 164]. Increased expression of IL-1 β in psoriatic epidermal cells, related to the activated KC-immunocyte in psoriasis. Psoriatic plaques express increased IL-1 β mRNA relative to non-lesional skin [161]. IL-1 β has been shown to induce the expression of adhesion molecules on various cell types and contributes to inflammatory responses. Activation of ERK, JNK, AP-1, and NFκB are leads to IL-1 β-induced ICAM-1 expression and leukocyte adhesion [165], thereby increase in ICAM-1 cause hyperproliferation of KC in psoriasis.

Normal KCs do not contain a biologically active form of caspases-1 [166], whereas in psoriatic epidermis, caspase-5 act as an upstream activator of caspase-1 [167]. Caspase-5 mRNA is induced by IFN-γ in vitro in both KCs and PBMCs and that this induction is most likely mediated through the NFκB pathway [168].

Mizutani et al. [169] found that IL-1 β levels in PBMC of psoriatic patients were decreased after 2 weeks of MTX treatment, due to suppression of KC paracrine system by MTX. Also, MTX effectively reduced IL-1 β levels in plasma and skin biopsies of psoriasis patients [153]. There are two possible mechanism of MTX decrease IL-1 β is elucidated above. One is direct mechanism and another one is indirect mechanism. The direct mechanism is as follows: MTX treatment inhibits the enzyme5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) transformylase (ATIC), which leads to accumulation of the substrate AICAR. The increased AICAR inhibit the enzymes, AMP deaminase and adenosine deaminase, which are essential for the catabolism of AMP and adenosine. This inhibitory action eventually increased adenosine in circulation. Thus, increased extracellular adenosine firmly increased cAMP in skin which finally inhibits the production of proinflammatory cytokine IL-1 β [163].

The indirect mechanism of MTX on reducing IL-1 β levels in psoriasis is mainly depend on reduction of infiltration of lymphocytes and monocytes in psoriatic dermis [162, 170]. MTX also decreased g the circulating and systemic levels of IL-1 β, by blocking the binding of IL-1 β to its respective receptor on monocytes, lymphocytes and granulocytes [171]. Studies have shown that IL-l α down-regulates IL-1 β via prostaglandin E_2 synthesis [172]. Therefore increase in IL-1 α levels by MTX, definitely downregulates IL-1 β by above said mechanisms. Another mechanisms of MTX reduced IL-1 β, its action on caspase-1 expression in lesional skin biopsy. This may due to IFN-γ reduction by MTX [173, 174], which leads to decrease in caspase-5 expression that causes reduction in caspase-1 expression.

7.1.2 Tumor necrosis factor alpha

TNF-α is a pivotal proinflammatory cytokine of the innate immune response and a key for skin inflammation [175]. TNF-α induce the expression of adhesion molecule ICAM-1 on KC through the mediation of p55 and ICAM-1 induces the infiltration of MNCs in the dermis, which promotes the development and progression of psoriasis vulgaris [176]. Thereby it causes hyperproliferation in psoriasis.

Many studies have shown that plasma concentration of TNF-α was significantly higher in psoriatic patients compared to the control group [35, 177, 178]. Johansen [179] showed increased TNF-α protein expression, but similar TNF-α mRNA levels, in lesional compared with nonlesional psoriatic skin, this results showed that TNF-α is regulated posttranscriptionally. Increased activation of MAPK-activated protein kinase 2 (MK2) is responsible for the elevated and posttranscriptionally regulated TNF-α protein expression in psoriatic skin. IL-1 β also amplified TNF-α protein expression by causing activation of p38 MAPK and MK2.

Action of MTX to overwhelm TNF activity is by suppressing TNF-induced nuclear factor-κB activation in vitro, in part related to a reduction in the degradation and inactivation of an inhibitor of this factor, IκBα, and probably related to the release of adenosine [180]. Gerards et al. [130] showed that Adenosine or adenosine receptor agonists inhibit production of TNF-α. MTX reduces TNF-α level and also reduces the adhesion molecules like E-selctin and ICAM-1, thereby it reduces TNF-α induced hyperproliferation in psoriasis. Decrease in TNF-α by MTX shows its anti-proliferative and anti-inflammatory effects.

7.1.3 Interferon-γ

IFN-γ is believed to be an important mediator in psoriasis. Accumulated evidence from both in vivo and in vitro studies show that IFN-γ is a critical element in the induction of KC hyperproliferation in psoriasis [172, 181]. Abdallah et al. [182] showed that serum IFN-γ is a psoriasis severity and prognostic marker.

Some authors showed that serum IFN-γ levels in psoriatic patients were 15-fold and in blister fluid 17-fold higher than those in the control group. They correlated with the clinical severity of psoriasis expressed as PASI score [183–186]. In lesional skin, expression of IFN-γ is induced by some cytokines like IL-12, IL-18, and IL-23 are termed as IFN-γ stimulator [187]. Apart from above said stimulator, IL-7 also indirectly increased IFN-γ levels in both the psoriatic skin and serum of psoriatic patients. Inflammatory mediator IL-7 interact with both IL-2 and IL-12, which indirectly induced the synthesis of IFN-γ in psoriatic skin [188].

MTX treatment effectively reduced IFN-γ levels in serum and skin biopsy of psoriatic patients. This may due to the action of MTX on reducing serum IL-7 levels and is found to correlate with disease in RA patients [20]. Similar to this result, studies have shown that MTX decreases the expression of IFN-γ in RA patients [135, 136]. Adenosine or adenosine receptor agonists inhibit production of IFN-γ [130]. In 1990, Nesher and Moore [189] proposed that MTX might inhibit polyamine synthesis in monocytes, thereby polyamines failed to restore IFN-γ production.

As we discussed earlier in this chapter about the MTX action on T cell cytolysis, the same concept is applicable in this mechanism. The primary source of activated T cell is IFN-γ [127]. MTX induce the cytolysis of activated T-cell which directly reduced the IFN-γ in psoriasis. Invitro studies showed that inhibition of NFκB led to the complete blockade of extracellular IL-17A, IL-22, IFN-γ, and TNF-α production in CD4+ cells [190]. It is well known that MTX directly inhibit NFκB [191]. Thus, MTX declines IFN-γ levels by inhibiting NFκB in psoriatic skin which control the hyperproliferation of KCs.

7.1.4 Transforming growth factor-β1

In the skin, TGF-β has been found to inhibit the growth of KCs but stimulate the growth of fibroblasts [192]. Plasma TGF-β1 is considered as a biomarker of psoriasis activity and treatment efficacy [193].

Gene and protein expression of TGF-β1 in lesional skin biopsies was found to be reduced compared to nonlesional skin biopsies [35, 97, 193]. Reduction in TGF-β1 in psoriasis may be due to increase in activated Akt levels. Activated Akt inhibit the phosphorylation of Smad2/3, is essential for TGF-β1 production [194, 195]. IFN-γ and NF-kappaB induces the expression of Smad7, an antagonistic Smad, which prevents the interaction of Smad3 with the TGF-β receptor [196, 197] leading to TGF-β1 production. Other factors that can suppress TGF-β1 productions are Th1 cytokines like TNF-α, IL-1, IL-6 [196]. All these factors could have lead to reduction in TGF-β1 levels in psoriasis. Reduction in TGF-β1 leads to increase in proliferation of KCs in psoriasis. Adhesiveness of T lymphocytes to dermal microvascular endothelial cells can be blocked by TGF-β1, so reduction of its expression and function may contribute to lymphocyte infiltration into psoriatic plaques [198].

MTX treatment causes overexpression of protein and mRNA level of TGF-β1 in lesional skin biopsy. Possible mechanism of MTX is accompanied by decreasing Ras methylation in psoriasis. This hypomethylation is accompanied by a mislocalization of Ras to the cytosol and a 4-fold decrease in the activation of Akt [199]. Decrease in Akt may lead to Smad 3 activation, which in turn increase TGF-β1 expression. Also MTX reduces Th1 cytokines like IL-1, IFN-γ, TNF-α, IL-6 [111, 135, 136, 188] which may lead to increase in TGF-β1 expression. Increase in TGF-β1 expression induced inhibition of KCs and is characterized by reversible retention of proliferation, or stopping of cell division cycle in the G1 phase [96]. Moreover, TGF-β causes transcriptional induction of K5 and K14 keratin genes [81]. Thus, TGF-β promotes the basal cell phenotype in stratified epithelia such as the epidermis, and that the effects of TGF-β are not anti-proliferative, but merely anti-hyperproliferative [78].

7.2 Keratin changes in psoriatic skin after MTX action

The pathogenesis of psoriasis is mainly depend on KCs and immune cells interaction. These interaction causes changes in the epidermal layers which destined the healthy skin

to lesional psoriatic skin is critical one. Due to the epidermal change, acanthosis is caused by disruption in the transition of KC to anucleate corneocytes. This disrupts transition leads to hyperproliferation and immatured differentiation of KC in psoriasis. Thus, an alternate or regenerative pathway is activated by abnormal immune response along with abnormal keratins in psoriatic epidermis [200].

7.2.1 Keratin 14

K14 is an important IF expressed in the basal KCs which decide the fate of epidermis by protecting it from any mechanical or chemical disturbances. Normallay, basal layer contains epidermal stem cells and TA cells that proliferate into KCs. The epidermal stem cells and TA cells expressed keratin K5/K14 which destined the cells to proliferate into KC [32].

In psoriatic skin biopsy, K14 expression was considerably higher than in normal epidermis [33–35]. However, MTX reduced the expressions of K14 in lesional skin biopies by increasing Phosphorylated form of p53 expression [201]. Studies have shown that p53 enhance the notch signaling [202], which leads to modulation and normalization of K14 expression to K10 differentiation marker. Thus, decrease in K14 expression leads to reduction in cell proliferation, decrease in phospho-Akt levels, increase in activated Notch1 levels, and increase in levels of KC differentiation markers [39] and also increased in p21 and p27 levels, which are known to be direct targets of Notch1 signals. Activation of p21 and p27 leads to cell cycle arrest [191, 203] which leads to inhibition of hyperproliferating KCs in psoriasis.

7.2.2 Keratin 10

When mitotically active basal epidermal KCs withdrawn from the cell cycle are committed to terminal differentiation, they switch off K14 expression and induce the expression of K10 [27]. K10 are the major structural proteins of the epidermis and belong to the large family of IF proteins [29, 41, 42]. K10 is also involved in the control of cell proliferation [43]. Decreased K10 protein and gene expression were observed in lesional psoriatic skin biospies [33–35]. Subsequently, hyperproliferation of KCs in psoriasis is mainly depend on the expression of K10. The possible mechanism is as follows, decrease in differentiation marker K10 leads to increased phosphorylation of Rb protein, which leads to increased epidermal cell cycle proteins, Cyclin D and E. The increased epidermal cell cycle proteins activate the phosphorylation of antiapoptotic protein Akt. Thus activation of Akt by decreased K10 leads to hyperproliferation of KCs in psoriasis [52].

E-cadherin is specific markers expressed in the endothelial cells are important for differentiation process. MTX promptly increased E-cadherin expression [201] which deliberately causes extracellular calcium concentration-dependent KC differentiation by increasing K10 in psoriatic skin. IL-22 which is considered to have an inhibitory effect on the expression of K10 [205], is reduced by MTX in psoriasis patients [206]. Also MTX increase c-fos expression which activate AP-1 transcription factor regulates K10 expression [207]. Thus MTX action on K10 expression proves the controlled differentiation in psoriatic KC.

The main therapeutic action of MTX is inhibiting DNA methylation by interfering folate metabolism [207]. Studies have shown that inhibition of DNA methylation directly increased transcription of K10 gene [208]. This shows the therapeutic action of MTX on psoriatic skin.

7.2.3 Keratin 16

Keratin 16 expressions, reduces the fraction of cells in G1 while increasing that in S phase [53]. Many authors also shown an upregulation of K6 and K16 [33–35] in psoriatic epidermis. Action of MTX on reducing Keratin 16 protein and mRNA expression in lesional skin biopsies is interesting. Ras signaling is also induces K16 in psoriasis. The action of MTX in decreasing K16 level is depends on the enzyme isoprenylcysteine carboxyl methyltransferase (Icmt). Inhibition of Icmt by MTX, indicates the link between antifolates and Ras. Icmt inhibition leads to decrease in carboxyl methylation of Ras [199], which induces EGF. Induced EGF directly decreased K16 expression. Overall mechanism indicates the action of MTX on K16 reduction. Apart from this, MTX also inhibits the IL-1, TNF-α levels [135, 153, 169], which also might leads to decrease in K16 expression in psoriasis. All these leads to inhibition of cell proliferation in psoriasis. Decrease in Keratin 16 by MTX indicates the strong anti-proliferative effect of MTX.

7.2.4 Keratin K17

Keratin K17, the myoepithelial keratin, which is not expressed in normal skin except for hair follicles, sweat and sebaceous glands and basal cells of the interfollicular epidermis in the scalp, is over-expressed in psoriatic epidermis [209, 210]. Keratin 17 is considered as a therapeutic target and marker of anti-psoriatic therapies used for the treatment of psoriasis [63, 64].

MTX substantially reduced abnormal K17 protein and gene expression in psoriasis by reducing circulatory inflammatory mediators like IL-22 [205] and IL-6 levels [111] in psoriasis patients. Many studies in Rheumatoid Arthritis patients showed that the production of IL-17 at the mRNA level and IFN-γ were reduced after MTX treatment [35, 135, 211]. Altogether MTX reduces K17 expression by decreasing IFN-γ inducers. Decrease in Keratin 17 by MTX indicates its therapeutic efficacy which helps to maintain the normal phenotype in KC.

8. Conclusion

When we put all these data and review together, we attain at a consistent outline for the action of keratins, growth factors and cytokines in psoriasis. MTX has been used as a effective agent in the treatment of psoriasis from the decades of 1960s. But still its mechanisms of clearing psoriatic remains ill-defined. In conclusion, we strongly thought that MTX inhibits the hyperproliferation of KCs by decreasing the levels of IL-1 and caspases-1 (activating signals), TNF-α, IFN-γ and by increasing deactivation signal through various effective pathway. Also it efficiently regulate abnormal keratins by upregulating K10 and downregulating K14, K16 and K17, thereby maintaining the normal phenotype in KC (**Figure 7**). Mutation in keratins filaments were also observed in psoriasis [31]. Thereby, understanding keratin functions and related regulatory mechanisms will help to design new therapeutic interventions for keratin-related skin diseases. We strongly concluded that MTX roles on controlling the KC-immunocyte cycle by activating important keratins and deactivating abnormal kertains showed its well-organized therapeutic effect in psoriasis patients.

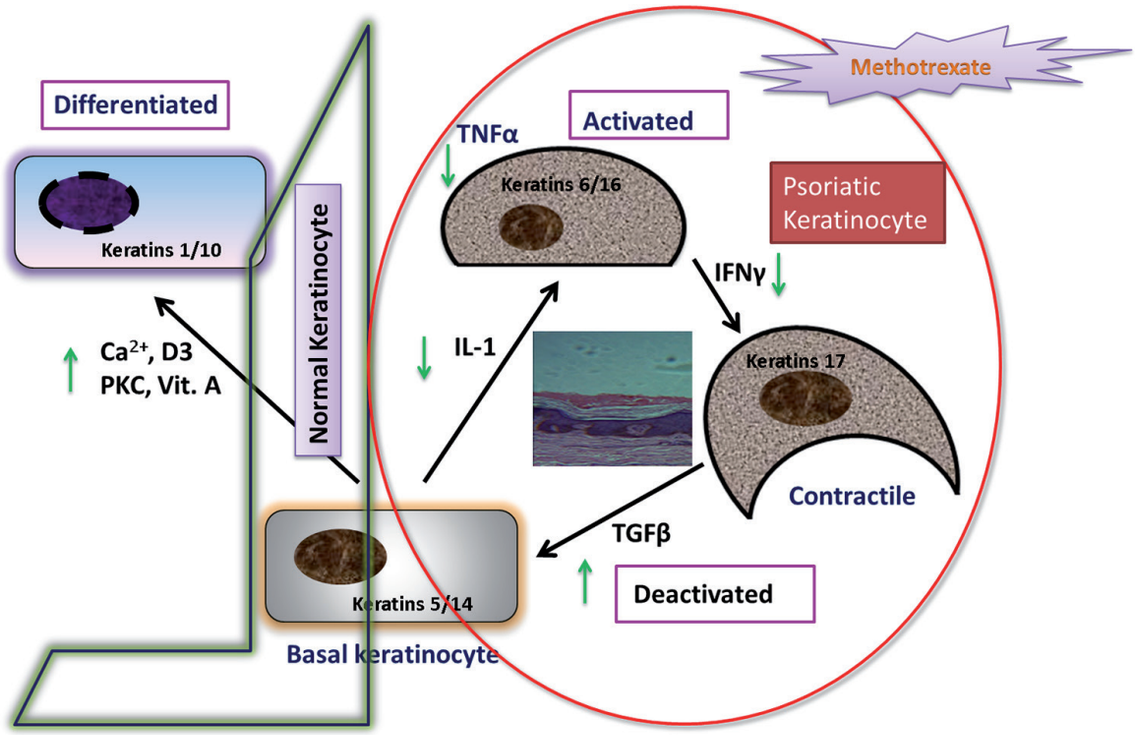


Figure 7.
Effect of MTX on KC activation cycle. MTX normalizing or reversing the phenotype of psoriatic KC but altering the inflammatory mediators as well as keratin proteins in KCs. Symbol ↑ indicates increase in psoriasis; symbol ↓ indicates decrease in psoriasis.

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Conflict of interests

The authors declare that they have no conflict of interests.

Abbreviation

KAC	keratinocyte activation cycle
KC	keratinocyte
IL	interleukin
MTX	methotrexate
TNF	tumor necrosis factor
IFN	interferon
TGF	transforming growth factor
K14	keratin 14
K10	keratin 10
K16	Keratin 16
K17	keratin 17

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