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Advance in the study on p38 MAPK mediated drug resistance in leukemia

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Abstract. – Leukemia is a group of malignant clone diseases of hematopoietic stem cells, while drug resistance (DR) has become the primary cause of chemotherapy failure. p38 mitogen-activated protein kinase (p38MAPK) signaling pathway induces cell activation, proliferation and apoptosis in response to extracellular stimuli. Recent studies have shown that p38 MAPK signaling pathway is closely associated with DR in leukemia. The present article reviews the relationship between p38 MAPK signaling pathway and DR in leukemia.

Key Words:
p38 MAPK, Leukemia, Drug resistance.

Introduction

Leukemia is a common hematologic malignancy in China, which causes the highest mortality rate in children and adults of age <35 years. Therapy of leukemia is individualized and comprehensive treatment composed primarily of chemotherapy; however, drug resistance (DR) has become one of the main causes for treatment failure. DR refers to a phenomenon whereby tumor cells once develop resistance to one chemotherapeutic agent, they also develop a cross-resistance to other agents that share similar chemical structures with but exert different mechanisms of actions from the agent. Some study has been reported that abnormality in p38 mitogen-activated protein kinase (MAPK) signaling pathway is closely related to the development and progression of malignant tumors[1]. In addition, a number of studies have shown that p38MAKP is also closely associated with multiple drug resistance (MDR) of leukemia cells. In the present study, advance in the study on the mechanisms of p38MAP mediated MDR against leukemia was reviewed in an effort to provide novel insights into the treatment of DR leukemias.

Mechanism of DR in Leukemia

Leukemia cells that are insensitive or resistant to chemotherapeutic agents are considered as DR. Two possible mechanisms underlying DR in leukemia have been proposed. One mechanism, which is recognized as primary DR, implies that original subtype of resistant cells aggregate, proliferate and eventually become major cell populations as sensitive cells have been selectively eliminated. The other mechanism, which is considered as secondary or acquired DR, entails that DR is caused by the alteration in cell characteristics induced by chemotherapeutic agents. Currently, studies have revealed the following mechanisms underlying DR in leukemia: (1) Drug efflux mediated by membrane glycoprotein, including P-glycoprotein (P-gp), MDR-associated protein, lung resistance related proteins (LRP), breast cancer related proteins (BCRP). These proteins transport anticancer drugs out of cells using the energy generated by ATP hydrolysis, thereby reducing the intracellular concentration of the drugs and leading to the development of DR[2,3]. (2) Mechanism mediated by enzymes, including DNA topoisomerase II (Topo II) and glutathione S-transferase gen (GST). Increased activities or elevated expressions of these enzymes can cause the emergence of DR. (3) Mechanism mediated by apoptotic genes, such as *Bcl-2* gene family and *p53* gene. (4) Abnormalities in multiple signaling pathways, such as MAPK signaling pathway and Hedgehog signaling pathway. (5) The effect of micro-environment. (6) Evasion of apoptosis. (7) Changes in drug target. (8) Enhanced repair of DNA damage in tumor cells. (9) Reduction in drug uptake in tumor cells[4,5]. Among these mechanisms, abnormality in signaling pathway is considered as a key mechanism causing DR. Meanwhile, recent studies have shown that p38 MAPK signaling pathway is related to the change in the expressions of DR related genes, including *Bcl-2, Top*

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II and *MDR1*. Therefore, study on p38 MAPK mediated mechanism underlying DR in leukemia is of great importance for reversing DR through p38 MAPK.

Components and Characteristics of p38 MAPK

MAPK signaling pathway, which is found in the majorities of mammalian cells, plays pivotal roles in transducing extracellular stimuli into cells and nuclei as well as in inducing cellular and biological activities (such as cell proliferation, differentiation and apoptosis)[6-8]. Multiple parallel MAPK signaling pathways have been discovered, which enables activation of one or multiple MAPK signaling pathways simultaneously by extracellular stimuli. Most common MAPK pathways include extracellular signal-regulated protein kinase (ERK1/2) pathway, C-jun NH2-terminal kinase (JNK) pathway, p38MAPK pathway, ERK3/ERK4 pathway and ERK5 pathway[9]. P38 MAPK signaling pathway plays important roles in stress responses such as inflammation and cell apoptosis. Moreover, apoptosis-mediated DR is considered as one of the mechanisms of DR. Over-expression of some genes can induce DR through altering the ability of leukemia cells in executing programmed death or changing apoptotic pathway.

P38 MAPK, as an important component of MAPK signaling pathway, was discovered by Brewster et al[10] when they were studying the impact of hyper-osmolarity on yeast in 1993. In 1994, Han et al[11] found, for the first time, the p38 MAPK encoding gene by stimulating mouse liver cells with hyper-osmolarity and endotoxin. Thereafter, Han et al[12] discovered three p38 isoforms, including p38β, p38γ and p38δ. To date, five isoforms of p38 MAPK have been discovered, including p38α (p38), p38β1, p38β2, p38γ and p38δ. P38 isoforms exhibit the following characteristics: (1) These isoforms respond differently to same stimulus. (2) Different isoforms functions selectively on substrates[13,14]. (3) Differences have been observed in the coupling different isoforms with different upstream kinases. MAPK kinase 6 (MKK6) can activate p38α, p38β2 and p38γ, whereas MKK3 can activate only p38α and p38γ[15]. (4) p38 MAPK distribution is tissue-specific[16]. P38 α is highly expressed in the white blood cells, the liver and the bone marrow, while p38 γ expresses exclusively in the skeletal muscle cells. (5) Different p38 isoforms mediate various biological responses in cells. For example, in Jurkat cells, p38 isoforms exert different effects on cell apoptosis in response to Fas antibodies and UV light. Expression of p38ß can reduce cell apoptosis whereas expression of p38α can enhance cell apoptosis in response to the same stimulus[17].

p38 MAPK Activation

P38 is located both in the nucleus and the cytoplasm without the presence of stimuli, but the locations of p38 differ in stimulated cells due to the functions of p38 on different substrates in response to various stimuli. These differences may be related to the intracellular location of p38, specific stimulation and the location of substrates under specific stimulus. MAPKs signaling pathway is activated through highly conserved kinase cascade consisting of three types of kinases, including MAP kinase kinase kinases (MAPKKK), MAP kinase kinases (MAPKK) and MAPK (figure 1). P38 MAPK is activated by either classical or non-classical pathway. Non-classical pathway is an important supplementary to classical pathway.

Classical Activation of p38 MAPK

P38 MAPK is classical activated through phosphorylation of threonin (Thr) and tyrosine (Tyr). Members of p38 MAPK family all have the Thr-Gly-Tyr (TGY) tripeptide motif that is located in the linker loop structure termed L12. This loop is the critical structure determining enzyme activity. Some residues in this loop structure develop a flexible loop termed the "phosphorylation lip" or "activation lip". Dual phosphorylation of Thr and Tyr by MAPKKs activates p38 MAPK[18] in response to various extracellular stimuli, such as oxidative stress, hypo-osmorality, UV radiation, hypoxia and circulation dilation, which activate MAPKKK, including ASK1, DLK, TAK1, MLK3 and MEKK3/4. Subsequently, MKK3/6 are activated and, in turn, activate p38 MAPK, which then activates STAT1, ELK-1, CHOP and ATF-2, leading to cell activation, proliferation and apoptosis[19] (Figure 1).

Non-classical p38 MAPK activation

P38 MAPK can also be activated via non-classical pathway, i.e., autophosphorylation of p38 MAPK, which is mediated by TAK1-binding protein1 (TAB1). TAB1 binds to Thr218 and Lle275 of p38α forming a complex, which leads to the autophosphorylation of p38α at Thr180 and Tyr182, and thereby activates p38α[20]. Inflammatory cell activating factors can activate

p38 MAPK through this pathway, which occurs mainly in B cells stimulated by LPS, TNF and CpG. Another non-classical activation of p38 MAPK occurs in T cells, where the Tyr323 of p38α and p38ß is activated by ZAP-70, which in turn, causes the autophosphorylation of Thr180 and Tyr182, leading to the activation of signaling pathway[21-23] (Figure 1).

Upon activation, p38 MAPK migrates from the cytoplasm to the nucleus and activates many substrates including transcription factors by specifically phosphorylated serine (Ser) and Thr, thereby regulating expression of related genes in respond to external stimuli.

Regulation of p38 MAPK

Regulation of MAPKs is very complex and regulatory proteins consist of small G protein of Rho family and TNF receptor-associated factor protein family (E3 ubiquitin ligase). The complex regulation of MAPK enables p38 signaling pathway to respond to various stimuli and integrates p38 MAPK pathway with other signaling pathways. P38 MAPK is regulated by multiple factors: (1) ATP-competitive inhibition. P38 transfers γ-phosphate to its substrate via ATP binding pocket at the junction between α and ß domains, thus achieving signal transduction. Hence, p38 can regulate p38 MAPK by either directly or indirectly competing binding site with ATP. (2) Since p38 MAPK activation is achieved by dual phosphorylation of Thr and Tyr, up-regulating the activity of phosphorylases on these two sites can inhibit p38 signaling pathway. (3) Stimulation of T cell receptor (TCR) can lead to autophosphorylation and activation of p38α and p38ß, in which TCR proximal tyrosine kinases, p56lck and Zap70 are essential. (4) Scaffold protein regulates p38 signaling pathway. Stimulated by hyper-osmorality, osmosensing scaffold for MEKK3 (OSM) binds to actin, Rac, and the upstream MEKK3 and MKK3, forming a complex and thereby regulating p38 activity[24]. Moreover, both upstream kinases and downstream substrates of p38 can serve as targets for p38 MAPK regulation.

Mechanisms of p38 MAPK Signaling Pathway Mediated DR

P38 MAPK may participate in development of DR through the following mechanisms, (1) The mechanism associated with cell apoptosis and autophagy. (2) p38 MAKP participates MDR by directly regulating the expression of DR related genes and proteins in leukemia. (3) Regulation of cell cycle by p38 MAPK. (4) The mechanism may be related to the extracellular stimuli for p38 MAPK activation.

p38 MAPK Mediated Regulation of Apoptosis and Autophagy

Apoptosis refers to programmed cell death of individual cells. The majority of anti-leukemia drugs exert anti-tumor effects by inducing apoptosis of leukemia cells. Abnormal expression of

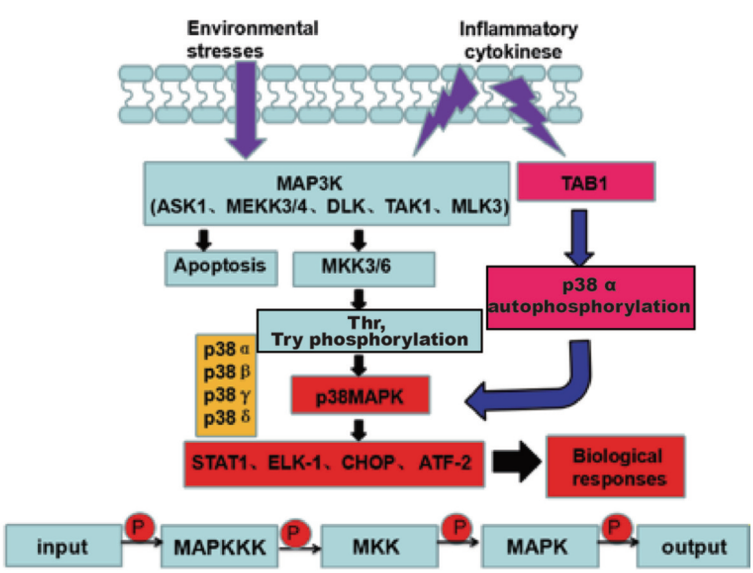

Figure 1. Mechanism of P38MAPK activation.

pro-apoptotic genes can enhance the anti-apoptotic capacity of leukemia cells and facilitate leukemia cells to evade apoptosis, thereby reducing the sensitivity of chemotherapeutic agents. Autophagy is a primary mechanism for eliminating mitochondrias and other organelles damaged by reactive oxygen species (ROS) and other factors as well as proteins with abnormal functions. Therefore, excessive autophagy is an important factor contributing to DR of tumor cells against cytotoxic drugs. In a variety of tumor models, autophagy is able to eliminate abnormal proteins, organelles and ROS, thereby leading to the emergence of DR[25-27]. Cell apoptosis and autophagy can be induced by extracellular stimuli such as chemotherapeutic agents and make cells respond differently to these drugs, thereby inducing the occurrence of DR. p38 MAPK is an important signaling pathway for the regulation of cell apoptosis and autophagy. Hence, p38 MAPK can participate in MDR of leukemia possibly by regulating cell apoptosis and autophagy.

Studies have shown that p38 MAPK is closely associated with induction of apoptosis[28,29]. Dong et al[30] have studied chronic myelogenous leukemia (CML) cells induced by imatinib and demonstrated that apoptosis of CML cells and the expression of apoptosis-related gene Bim are achieved by p38 MAPK mediated Histone 2AX (H2AX) phosphorylation. The study indicated that p38 MAPK activation may be accompanied by H2AX response, and H2AX phosphorylation can be blocked by p38 siRNA and its inhibitor. All of these data suggest that H2AX phosphorylation regulated by p38 MAPK pathway is involved in the regulation of Bim gene expression and apoptosis of K562 cells.

Moreover, autophagy has been shown to be closely related to p38 MAPK pathway. Autophagy can be suppressed thereby increasing cytotoxicity by increased oxidative stress, induction of mitochondrial dysfunction and activation of JNK and p38 MAPK[31]. In addition, p38 MAPK signaling pathway exerts different effects on autophagy of different cell lines. In NB4 cells, p38 MAPK is released and undergoes autophosphorylation through inhibiting the expression of heat shock protein (Hsp)90, thereby transferring external stimuli to downstream transcription factor ATF4 and down-regulating autophagy. In Jurka cells, p38 MAPK activated by MKK3/6 cascade facilitates the binding of activating transcriptional factor4 (ATF4) to microtubule-associated protein1lightchain3B (map1lc3b) promoter through the mediation of eukaryotic initiation factor 4E (eIF4E), thereby enhancing autophagy of the cells. Studies[32,33] have shown that JNK and p38 MAPK pathways are likely to control the balance of autophay and apoptosis. Jiang et al[34] suggested that p38 MAPK activation plays important roles in apoptosis of leukemia cells and the process of autophagy. MKK3/6-p38MAPK-eIF4E cascade activates ATF4, thereby regulating the expression of apoptosis- and autophagy-related genes. Up-regulation of ATF4 expression can induce the transcription of apoptosis-related gene CCAAT/enhancer-binding protein-homologous protein (CHOP) and autophay-related gene map1lc3b. Hence, enhancing cell apoptosis and autophagy induced by stress-related factors can be used as a therapeutic strategy in the treatment of leukemia. Moreover, autophagy induction can enhance cell apoptosis under certain conditions.

p38 MAPK Regulation of the Expression of DR-related Genes and Proteins

P38 MAPK has been shown to participate in the development of MDR in leukemia cells by regulating the expression of DR-related genes and proteins[35]. Kim et al[36] demonstrated that mercury can induce the expression of DR-related genes mediated by p38 MAPK signaling pathway. Huang et al[37] showed that activin A induction of erythroid differentiation mediated by p38 signaling pathway can enhance the sensitivity of K562 CML cells to imatinib. In addition, they also confirmed that suppression of p38 by its inhibitor or short hairpin RNA accompanied by the combination therapy of activin A and imatinib can regulate cell apoptosis as well as growth inhibition, reducing erythroid differentiation. Besides, p38 inhibition SB-203580 can restore activin A-inhibited MDR-1 expression. The study also found that inhibition of NHE1 activity significantly reduces P-gp expression in resistant cells, increases the accumulation of rhodamine 123 and doxorubicin in CML cells during progressive phase of the disease and enhances cell sensitivity to imatinib, all of which involve p38 MAPK signaling pathway. Taken together, p38 MAPK pathway may participate in the emergence of and be positively correlated with MDR of leukemia cells.

However, the reactions of p38 isoforms are different in response to same stimulus and so are the biological effects mediated by these isoforms, resulting in a variety of functions mediated by p38 MAPK signaling pathway induced by

different extracellular stimuli. Currently, effect of p38 MAPK pathway on MDR-related genes and proteins remains controversial. Relevant evidence showed that p38 MAPK is negatively correlated with DR of leukemia cells. Chen et al[38] demonstrated that p38 inhibitor SB20219 significantly reduces the mRNA expression of MDR1 and P-gp in K562/DOX cells, which implies that p38 MAPK signaling pathway can enhance the activity of P-gp whereas p38 inhibitor can reduce its activity in K562/DOX cells.

Cell cycle Regulation by p38 MAPK Signaling Pathway

Cell cycle regulation is the theoretical foundation for chemotherapy of leukemia. Multiple chemotherapy drugs exert anti-tumor effects through blocking the progression of various phases of cell cycle. Methotrexate, a commonly used chemotherapeutic agent, selectively affects S phase and G1/S progression. Cytarabine primarily acts on S phase and delays or partially blocks the progression of G1 to S phase, thereby arresting cells in G1 phase. P38 MAPK has been shown to regulate G2/M and G1/S cell cycle checkpoints in response to stress-induced DNA damage. Up-regulating cell cycle checkpoint facilitates DNA repair and hence reduces cell sensitivity to chemotherapy agents. P38 MAPK can phosphorylate Ser15 and Ser392 of p53, activate and stabilize p53 through inducing target genes Gadd45α and p21, thereby up-regulating G2/M checkpoint and directly or indirectly inhibiting the activation of G2/M-promoting cdc2/cyclin B complex[39]. Miyoshi et al[40] found that benzyl isothiocyanate (BITC)-induced G2/M cell cycle arrest and cell apoptosis in Jurkat cells is achieved through p38 MAPK signaling pathway, which is evidenced by the finding that only p38 MAPK inhibitor significantly reduces inactive phosphorylated Cdc2 protein and the G2-M arrested cell number.

Correlation Between MDR and Extracellular stimuli for p38 MAPK

It is reported that p38 MAPK-inducing extracellular stimuli, including oxidative stress, hypo-osmorality, UV radiation and hypoxia, have a certain relationship with MDR of cells, in a way that these stimuli induce the emergence of MDR in leukemia cells. Aggressive growth of tumor cells results in a microenvironment featured by hypoxia and acidity. Hypoxia enhances the expression of MDR genes. Hypoxia inducible factor-1 (HIF-1) can affect hypoxic area of tumor through activating *MDR1* gene and the encoded P-gp protein enhances DR of tumor cells in hypoxic area by mediating efflux of chemotherapy drugs. In addition, *in vitro* hypoxia treatment specifically suppresses DNA replication in S phase. However, the majority of anti-tumor drugs function primarily on cells undergoing rapid division. Hence, hypoxia can lead to the reduction in the sensitivity of tumor cells to anti-tumor drugs. Furthermore, the change of pH value in hypoxic tissues (pH=7.05 in hypoxic tissues *vs*. pH=7.3 in normal tissues) can affect the extracellular and intracellular distribution of anti-cancer drugs[41,42]. In addition, UV radiation causes DNA mutation and induces the expression of novel genes or inactivates existing genes, which result in the generation of novel enzymes or structural proteins using alternative pathways, thereby inducing the alteration of drug sensitivity.

Conclusions

Reversing DR plays a vital role in chemotherapy of leukemia and study on mechanisms of DR is essential for conquering DR in leukemia[43]. Complexity of mechanisms underlying DR limits the advance of leukemia therapy, however, researches on the relationship between p38 MAPK pathway and DR will provide novel therapeutic targets for the treatment of resistant leukemia. Exploring key targets in p38 MAPK pathway that are involved in DR of leukemia and subsequently specific inhibition of multiple targets will significantly improve cure rate for leukemia, bringing new hope to the treatment of chemotherapy-resistant leukemia.

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