Emodin Inhibits Resistance to Imatinib by Downregulation of Bcr-Abl and STAT5 and Allosteric Inhibition in Chronic Myeloid Leukemia Cells

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Emodin-resistance is a significant concern for Bcr-Abl-positive chronic myelogenous leukemia (CML) treatment. Emodin, the predominant compound of traditional medicine rhubarb, was reported to inhibit the multidrug resistance by downregulating P-glycoprotein of K562/ADM cells with overexpression of P-glycoprotein in our previous studies. In the present study, we found that emodin can be a potential inhibitor for the imatinib-resistance in K562/G01 cells which are the imatinib-resistant subcellular line of human chronic myelogenous leukemia cells with overexpression of breakpoint cluster region-abelson (Bcr-Abl) oncoprotein. Emodin greatly enhanced cell sensitivity to imatinib, suppressed resistant cell proliferation and increased potentiated apoptosis induced by imatinib in K562/G01 cells. After treatment of emodin and imatinib together, the levels of p-Bcr-Abl and Bcr-Abl were significantly downregulated. Moreover, Bcr-Abl important downstream target, STAT5 and its phosphorylation were affected. Furthermore, the expression of Bcr-Abl and signal transducers and activators of transcription 5 (STAT5) related molecules, including c-MYC, MCL-1, poly(ADP-ribose)polymerase (PARP), Bel-2 and caspase-3, were changed. Emodin also decreased Src expression and its phosphorylation. More importantly, emodin simultaneously targeted both the ATP-binding and allosteric sites on Bcr-Abl by molecular docking, with higher affinity with the myristoyl-binding site for enhanced Bcr-Abl kinase inhibition. Overall, these data indicated emodin might be an effective therapeutic agent for inhibiting resistance to imatinib in CML treatment.

Key words chronic myeloid leukemia; imatinib-resistance; emodin; breakpoint cluster region-abelson tyrosine kinase; signal transducer and activator of transcription 5; allosteric inhibition

INTRODUCTION

Chronic myelogenous leukemia (CML) is a malignant proliferative disorder that arises out of a chromosomal translocation (9; 22),1,2 which causes the formation of constitutively active breakpoint cluster region-abelson (Bcr-Abl) tyrosine kinase. The dysregulated activity of tyrosine kinase Bcr-Abl results in the activation of cell signaling pathway,3 which is recognized to be the therapeutic target for CML.4 Imatinib, the tyrosine kinase inhibitor (TKI), has been always used clinically as the first-line therapy in CML treatment.5 However, the acquisition of imatinib (IM) resistance is a critical event to some patients.6,7 Although the second-generation and third-generation inhibitors, such as nilotinib,8 dasatinib9 and ponatinib10 were developed, it is not desirable to resolve the event completely.

Clinical outcome-based TKI resistance can be explained by several Bcr-Abl-dependent and independent molecular mechanisms, including drug influx and efflux, increased Bcr-Abl expression, Bcr-Abl mutations, hyper-activation of alternative protooncogenic signaling network. Though TKI-binding site mutation is the most frequent mechanism of resistance, which alters the conformation of Bcr-Abl and reduces its affinity to TKIs, amplification of Bcr-Abl was also observed in animal models11 and CML patients.12 Indeed, several recent studies hypothesized Bcr-Abl overexpression is critical in the development of mutations, which represented a first step to induce mutation and resistance.13 Besides, it seemed that Bcr-Abl overexpression enhanced self-renewal of CML stem cells.14 In other cases, activation of prooncogenic signaling pathways and molecules, such as signal transducers and activators of transcription 5 (STAT5) and Src kinases, have been described as avoiding TKI treatment and contributing to CML resistance.

Recently, in order to overcome TKI resistance, one effective approach has been proposed to apply combinations of different targeted drug and cover a larger spectrum of relevent targets. Ascinibin, an allosteric Bcr-Abl inhibitor, was highlighted as a highly active additional agent for the clinical treatment of Bcr-Abl-positive CML. Drug combination with ascinibin and TKI, might provide great opportunities for more rapid and deeper remissions, and further addressing the issue of CML resistance.15

Emodin (3-methyl-1,6,8-trihydroxyanthraquinone) (Fig. 1A) is a predominant compound in the root of *Rheum palmatum* L. and *Polygonum multiflorum*,16,17 with multiple pharmacological actions of purgative, hepatoprotective, vasorelaxent, antibacterial, anti-inflammatory, immunosuppressive, and cardiotonic effects in nature.18–23 Recent studies have also displayed its potential anti-cancer capabilities of suppressing proliferation, inhibiting tumor resistance, inducing apoptosis, and sensitizing cancer chemotherapy in a diversity of malignant human cancers in vivo and in vitro.24–30 Emodin possessed a synergistic growth-inhibitory effect with AZT via decreasing the expression of P-glycoprotein tightly in K562/ADM, doxorubicin-resistant variant of K562 cells.31 Also, in our previous report, emodin reversed the resistance of K562/ADM cells to
adriamycin by a decrease of P-glycoprotein protein expression and competition of the R transport site on P-glycoprotein as the potential substrate of P-glycoprotein. However, there are few studies on whether emodin would inhibit resistance to imatinib in K562/G01 cells with resistant mechanism of Bcr-Abl overexpression and high tyrosine kinase activities, which is imatinib-resistant variant of K562 cells. In the current study, we investigated the effects of emodin inhibiting imatinib-resistance in K562/G01 cells and the molecular mechanism involved with Bcr-Abl and related targets.

MATERIALS AND METHODS

Chemicals and Reagents Emodin was purchased from the National Institutes for Food and Drug Control of China (Beijing, China); and imatinib mesylate was obtained from Sigma-Aldrich Co. (St. Louis, MO, U.S.A.); both compounds, with purity of 98% (HPLC), were prepared in dimethylsulfoxide (DMSO, Sigma-Aldrich) at a stock concentration of 100 mM, stored at −20°C, and then diluted further as needed. Roswell Park Memorial Institute-1640 (RPMI-1640), fetal bovine serum (FBS), penicillin, streptomycin were products of Gibco BRL (Rockville, MD, U.S.A.). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), dihydrochloride (2-(4-amidinophenyl)-6-indolecarbamidine dihydrochloride, DAPI) and other reagent grade chemicals were purchased from Sigma-Aldrich Co. Annexin V-APC apoptosis detection kit was obtained from ebioscience Inc. (San Diego, CA, U.S.A.). RIPA lysis buffer was purchased from Beyotime (Shanghai, China). Protease inhibitor cocktail was the product of F. Hoffmann. La Roche, Ltd. (Basel, Switzerland). The primary antibodies against to Bcr-Abl, phospho-Bcr-Abl, STAT5, phospho-STAT5, Src, phospho-Src (Tyr416), poly-ADP ribose polymerase (PARP), MCL-1, c-MYC, Bcl-2 and caspase-3 were obtained from Cell Signaling (Beverly, MA, U.S.A.). The β-actin and horseradish peroxidase (HRP)-linked secondary antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, U.S.A.).

Cell Lines Human chronic myeloid leukemia imatinib-resistant subcellular line K562/G01 was obtained from Institute of Hematology of Chinese Academy of Medical Sciences (Tianjin, China). The imatinib-resistant K562/G01 cell line was obtained by incubating K562 cells with increased imatinib concentrations for ten months. All CML cell lines were cultured in RPMI 1640 media supplemented with 10% fetal bovine serum containing 100 U/mL penicillin and 100 mg/mL streptomycin at 37°C under 5% CO₂. All cells used in experiments were in the linear phase of growth.

Cell Viability Assay As described previously, cell viability was measured by MTT assay for cytotoxic evaluation of emodin, imatinib alone or the combination. After corresponding treatment, 20 μL of freshly prepared MTT (5 mg/mL in phosphate buffered saline (PBS) solution) was added to each well and incubated for 4h at 37°C. The resulting formazan crystals were dissolved in DMSO (150 μL/well) by shaking for 10min until no particulate matter was observed. The absorbance was measured at 570 nm on a Bio-Rad Model/550 microplate reader (Hercules, CA, U.S.A.). The cell inhibition
To investigate the cytotoxic effects of imatinib in combination with emodin, K562/G01 cells were treated with imatinib, emodin alone and a combination, respectively. The cell viability was measured by MTT assay, and the cell inhibition rates and RF values were calculated. The K562/G01 cell line used in the experiment, was resistant to imatinib with 14.42-fold compared to K562 cells. After K562/G01 cells were handled with various concentrations of emodin (0–80 μM) alone for 24, 48, or 72 h, the cell viability was slightly affected by low concentrations of emodin (0 – 40 μM) (cell viability >70%, Fig. 1B). However, emodin at the concentration of 80 μM for 72 h markedly inhibited the growth of K562/G01 cells (cell viability <50%). Considering the solubility of emodin in cultured medium and the toxicity of emodin itself on cell growth, low concentrations of emodin (0–40 μM) were co-administrated with imatinib to K562/G01 cells for 72 h, to further evaluate the inhibitory effects of imatinib in combination with emodin. See from Fig. IC, the values of imatinib IC50 were reduced with increasing concentrations of emodin in a dose dependent manner, and the RF value reached 10.01 at emodin concentration of 40 μM, which suggested that the treatment with emodin might activate or suppress signal pathways to improve the inhibitory effects of imatinib on cell growth. The present findings indicated that emodin was potential of inhibiting imatinib-resistance in K562/G01 cells.

**Docking Study**

The molecular docking analysis was performed for further evaluation of the binding modes of emodin with Bcr-Abl refined from the original crystallographic data (PDB ID: 5MO4). The molecular docking analysis was performed in accordance with previously reported methods, with minor modifications. In Molecular Operating Environment (MOE) program (Version 2008, Chemical Computing Group Inc, Montreal, QC, Canada), the myristoyl-binding pocket was formed in accordance with previously reported methods, with minor modifications. In MOE, the donor hydrogen atoms were added, gasteiger partial charges were computed, solvent molecules were removed and energy minimization was performed by the Merck Molecular Force Field (MMFF94) forcefield. Emodin was docked into the Abl kinase active sites via means of the default triangle matcher algorithm from MOE. The London dG scoring function of the MOE was chosen to rank the docking poses of hits (lower scores indicate more favorable poses).

**Statistical Analysis**

Data were presented as mean ± standard deviation (S.D.) of at least three independent experiments. Significant differences were analyzed by Student’s t-test or ANOVA using GraphPad Prism software (Version 6.01). A value of less than 0.05 (p < 0.05) was considered to be statistically significant.

**RESULTS**

**Emodin Enhances the Inhibitory Effects of Imatinib on Viability of K562/G01 Cells**

To investigate the cytotoxic effects of imatinib in combination with emodin, K562/G01 cells were treated with imatinib, emodin alone and a combination, respectively. The cell viability was measured by MTT assay, and the cell inhibition rates and RF values were calculated. The K562/G01 cell line used in the experiment, was resistant to imatinib with 14.42-fold compared to K562 cells. After K562/G01 cells were handled with various concentrations of emodin (0–80 μM) alone for 24, 48, or 72 h, the cell viability was slightly affected by low concentrations of emodin (0 – 40 μM) (cell viability >70%, Fig. 1B). However, emodin at the concentration of 80 μM for 72 h markedly inhibited the growth of K562/G01 cells (cell viability <50%). Considering the solubility of emodin in cultured medium and the toxicity of emodin itself on cell growth, low concentrations of emodin (0–40 μM) were co-administrated with imatinib to K562/G01 cells for 72 h, to further evaluate the inhibitory effects of imatinib in combination with emodin. See from Fig. IC, the values of imatinib IC50 were reduced with increasing concentrations of emodin in a dose dependent manner, and the RF value reached 10.01 at emodin concentration of 40 μM, which suggested that the treatment with emodin might activate or suppress signal pathways to improve the inhibitory effects of imatinib on cell growth. The present findings indicated that emodin was potential of inhibiting imatinib-resistance in K562/G01 cells.

**Emodin Enhances Imatinib-Induced Cell Apoptosis of K562/G01 Cells**

To further evaluate the effects of emodin on imatinib-induced apoptosis, K562/G01 cells were incubated with emodin, imatinib or a combination for 72 h, respectively. Considering that the maximal fluorescence spectrum of emodin was between 515 and 525 nm, Annexin V-APC and DAPI were selected for the apoptosis assay to avoid fluorescence interference of emodin. As shown in Fig. 2, 20 or 40 μM emodin or 1 μM imatinib slightly increased the apoptotic proportions of K562/G01 cells with the apoptotic rates of 8.44, 12.39, and 8.83%, respectively. In contrast, K562/G01 cells treated with 20 or 40 μM emodin combined with 1 μM imatinib showed significant apoptosis with apoptotic percent of 23.9 and 45.5%, respectively (p < 0.01). These results illustrated that emodin significantly increased the imatinib-induced apoptosis of K562/G01 cells in a dose-dependent manner, which supported that emodin could potentially inhibit imatinib-resistance in K562/G01 cells.

**Emodin Enhances the Inhibitory Effects of Imatinib by Downregulation of Bcr-Abl and STAT5 Expression and Phosphorylation in K562/G01 Cells**

The oncprotein Bcr-Abl, the therapeutic target of imatinib, constitutively activates tyrosine kinase activity, thereby activating cell signaling pathways in CML. To evaluate the effects of imatinib and emodin on the levels of Bcr-Abl expression and phosphorylation in K562/G01 cells, Western blot analysis was performed. After the K562/G01 cells were incubated with imatinib or emodin alone for 72 h, emodin or imatinib only slightly decreased the Bcr-Abl expression and phosphorylation compared with the control group in these cells. Nevertheless, the levels of Bcr-Abl expression and phosphorylation were clearly decreased dose-dependently, when K562/G01 cells were treated with a combination of imatinib and emodin (Fig. 3A).

One family of transcription factors, STAT5, is a critical signal transducer activated by tyrosine kinases in hematologic cancers. Recently, STAT5 is known as an attractive target
to overcome resistance to Bcr-Abl kinase inhibitors, even in the presence of the T315I Bcr-Abl mutation of CML cells. Thus, the expression and phosphorylation of STAT5 were examined in K562/G01 cells treated with imatinib, emodin and a combination. In Fig. 3B, the STAT5 protein expression and phosphorylation were not influenced in K562/G01 treated with 1 \( \mu \)M imatinib and emodin alone for 72 h, which might be related to the mechanism that Bcr-Abl was only slightly effected by 1 \( \mu \)M imatinib and emodin. Moreover, compared with each single drug, the combination of imatinib and emodin significantly reduced the expression and phosphorylation levels of STAT5 in a dose dependent manner, which was consistent with the trend of Bcr-Abl expression and phosphorylation. Consequently, it is reasonable to conclude that emodin could effectively inhibit the imatinib-resistance of K562/G01 cell line by downregulation of Bcr-Abl and downstream STAT5 expression and phosphorylation.

**Emodin Enhances the Inhibitory Effects of Imatinib on Bcr-Abl and STAT5 Downstream Signaling in K562/G01 Cells**

To further investigate the underlying mechanism by which emodin enhanced the inhibitory effects of imatinib on resistance in K562/G01 cells, the alterations in Bcr-Abl and STAT5 signaling pathway related with cellular proliferation and apoptosis, including downstream PARP, MCL-1, c-MYC, Bcl-2 and caspase-3, were investigated by Western blot analysis. MCL-1 and c-MYC are the target gene products of Bcr-Abl/STAT5 signaling pathway. As expected, the expression levels of the anti-apoptotic protein MCL-1 and the oncoprotein c-MYC were slightly downregulated in K562/G01 cells exposed to imatinib and emodin alone, while these two downstream signal proteins showed a significant decrease in their expressions after 72 h of a combination of imatinib and emodin, in correlation with the decrease in protein expression and phosphorylation of Bcr-Abl and STAT5 (Figs. 4A, B). Apoptosis induced by the combination of imatinib and emodin was further confirmed by PARP cleavage, the activation of caspase-3 and the downregulation of Bcl-2 (Figs. 4A, B). These results suggested that Bcr-Abl and STAT5 related signaling mediated the combined inhibitory effects of imatinib and emodin.

**Emodin Enhances the Inhibitory Effects of Imatinib on Src Expression and Phosphorylation in K562/G01 Cells**

Src kinases play a role in proliferation of Bcr-Abl expressing cell lines. Overexpression of Src kinases has been identified...
as potential mediators of leukemogenesis induced by Bcr-Abl, and implicated in imatinib-resistance, which cooperates with Bcr-Abl kinase to activate STAT5 in CML cell transformation.\textsuperscript{40,41}) To assess the effects of emodin on Src expression and phosphorylation in K562/G01 cells, Western blot analysis was performed with specific antibodies to Src and p-Src (Tyr416). In cells treated by 1\textmu M imatinib and various dosages of emodin alone for 72 h, total Src expression and phosphorylation of Y416 were only partially affected when emodin concentration was 40µM, while Src and p-Src were effectively suppressed in response to a combined treatment of emodin and imatinib (Figs. 4C, D). These results indicated that the mechanism of emodin enhancing the inhibitory effects of imatinib was related with suppression of Src expression and phosphorylation.

Molecular Docking Analysis According to the previous reports, the Bcr-Abl fusion protein has two drug target sites, including the ATP-binding site and the retained myristoyl-binding pocket, which were successfully used for the development of the TKIs and the allosteric inhibitors.\textsuperscript{15}) To explore the modes of emodin interacting with Bcr-Abl on these two drug target sites in detail, molecular docking was performed between emodin and the X-ray crystal structure of Ab1.\textsuperscript{36}) Based on the results of molecular docking, emodin bound to the myristoyl-binding pocket with the binding free energy of $-17.995$ kcal/mol. As shown in Fig. 5A, at the allosteric site, emodin formed hydrogen–bonding interactions with residues Tyr454, Glu481, Ala356 and Val525. By contrast, it bound to the ATP-binding site with the less negative binding free energy of $-13.159$ kcal/mol than that of the myristoyl-binding pocket, though there were some hydrogen–bonding interactions with Gly246, Lys253, Asn115, and His114 at the ATP-binding site (Fig. 5B) (the less negative binding free energy of emodin with Bcr-Abl binding site represents the easier interaction between emodin and Bcr-Abl protein). In summary, emodin could bind to the two target sites of Bcr-Abl protein, while its interaction with the myristoyl-binding pocket had a much higher affinity than the ATP-binding site, which elucidated well why the combination of emodin and imatinib with no individual efficacy could synergistically inhibit the resistant Bcr-Abl high expression in K562/G01 cells. Comprehensive crystallographic and biochemical analyses have fully proved that transient binding at the ATP-binding site facilitates binding at the myristoyl-binding pocket, which in turn further stabilizes the binding at the ATP-binding site.\textsuperscript{15}) Based on the above mechanism, emodin at low concentrations restored imatinib's effectiveness against currently resistance induced by overexpression Bcr-Abl.

DISCUSSION

The success of imatinib mesylate in the treatment of CML has been a landmark event in clinic therapeutics.\textsuperscript{42}) Compared with the second- and third-generation TKIs, imatinib exhibits a lower risk of adverse effect. Two independent studies de-
signed to assess the long-term prognosis of patients receiving imatinib treatment showed that among patients achieving a complete cytogenetic response, CML-related deaths were rare and their survival rates were not different from those of the general population.\textsuperscript{35,40} Regrettably, the occurring of resistance limits the clinic application of imatinib to a certain degree, with several important mechanism of intrinsic stem cell resistance.\textsuperscript{41} drug influx and efflux, increased Bcr-Abl expression, Bcr-Abl mutations, hyper-activation of alternative protocarcinogenic signaling network.\textsuperscript{42} Overexpression of Bcr-Abl is one critical key of inducing mutation and resistance and enhancing self-renewal of CML stem cells.\textsuperscript{15,43} However, it has not been provided how to resolve the CML resistant barrier of increased Bcr-Abl expression effectively. Many studies indicated that emodin, a major component in rheum, showed multiple effects in cancer therapy, including tumor growth inhibition, apoptosis induction, toxicity sensitization and migration inhibition.\textsuperscript{18–30}\textsuperscript{54} Docking analysis provided much more information to understand the mechanism better by which combining emodin might explain that emodin inhibited the imatinib-resistance and sensitizet imatinib-induced apoptosis in drug-resistant CML cells of K562/G01 with Bcr-Abl expression by inhibiting Bcr-Abl/STAT5/c-MYC/MCL-1 oncogenic pathway and Sre expression and phosphorylation.

Recent paper showed that 3'-azido-3'-deoxythymidin cooperate with emodin in inhibiting the growth of adriamycin-resistant human chronic myelogenous leukemia (K562/ADM) cells.\textsuperscript{31} We previously reported that emodin inhibited adriamycin resistance in K562/ADM cells by inhibiting P-glycoprotein protein expression and competing the R transport site on P-glycoprotein.\textsuperscript{52} In the current study, our data demonstrated that emodin was greatly active across imatinib-resistant K562/G01 CML cells and gave significant inhibition of resistance to imatinib in a time- and dose-dependent manner. Moreover, emodin enhanced imatinib-induced apoptosis in K562/G01 cells in a dose-dependent manner. These results that the combination of emodin and imatinib could effectively inhibit the proliferation of TKI-resistant cells and enhance the apoptosis of TKI-resistant cells induced by imatinib, confirmed that emodin might be an interesting candidate for treatment of imatinib-resistance.

Several literatures indicated that STAT5 activation has been shown to be absolutely essential for leukemic cell survival and tyrosine kinase inhibitor-resistance of CML cells.\textsuperscript{46,47} High level of STAT5 leads clearly to a TKI-resistant phenotype and protects leukemic cells from TKI toxicity.\textsuperscript{45} As for Bcr-Abl/STAT5 signaling pathway, Bcr-Abl promotes activation of STAT5 downstream target genes by directly inducing a tyrosine-phosphorylation of STAT5. Moreover, STAT5 has been identified as an additional, important regulator that modulates the sensitivity of Bcr-Abl cells against TKIs.\textsuperscript{48} Accordingly, Bcr-Abl and downstream STAT5 protein expression and phosphorylation were investigated in K562/G01 cells treated with emodin, imatinib alone or a combination. One micromole imatinib, 10 or 20µM emodin did not clearly block expression and phosphorylation of Bcr-Abl and STAT5, and 40µM emodin produced only a slight effect on them. Only the combination of both compounds resulted in obvious inhibition of expression and phosphorylation of both Bcr-Abl and STAT5, which might explain that emodin inhibited the imatinib-resistance of K562/G01 cell line by downregulating Bcr-Abl/STAT5. Functional consequences of Bcr-Abl/STAT5 pathway inhibition by the co-treatment of imatinib and emodin were shown by decreased protein contents of c-MYC and MCL-1, which are connected with Bcr-Abl/STAT5 signaling pathway.\textsuperscript{37,49,50}\textsuperscript{50} Importantly, overexpression of c-MYC has been implicated in Bcr-Abl mediated the malignant transformation of CML. It is exemplified that Bcr-Abl can regulate c-MYC expression through distinct mechanisms.\textsuperscript{50} Furthermore, MCL-1 transcription is dependently regulated by c-MYC to hamper the cell apoptosis.\textsuperscript{51} Besides, our study demonstrated that combination of imatinib and emodin diminished the protein content and Tyr416 phosphorylation of Src, which belongs to a family of structurally related kinases in the Src family kinases (SFKs). In CML, SFKs cooperate with Bcr-Abl kinase to active STAT5 and are involved in imatinib-resistance.\textsuperscript{52} Therefore, in our present work, suppression of Bcr-Abl/STAT5/c-MYC/MCL-1 pathway and Scr by combination of imatinib and emodin contributed to inhibition of imatinib-resistance and enhancement of imatinib-induced apoptosis in K562/G01 cells.

Crystallographic analysis of Bcr-Abl protein highlights a two-lobe catalytic domain (N- and C-lobes towards N- and C-terminus of the sequence, respectively), besides a P-loop residue, a gatekeeper residue of Thr315 and a DFG (aspartic acid (Asp)-phenylalanine (Phe)-glycine (Gly)) motif, which are the ATP-binding sites targeted by all Bcr-Abl TKIs.\textsuperscript{53} Additionally, the retained myristoyl-binding pocket within the Bcr-Abl kinase domain represents a amenable site to allosteric inhibiotion, and the allosteric inhibitors of Bcr-Abl were designed to sepecifically inhibit Bcr-Abl kinase and its downstream signaling.\textsuperscript{54} Docking analysis provided much more information to understand the mechanism better by which combining emodin

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**Fig. 5. The Ribbon Diagrams of Docking Emodin with Bcr-Abl (Abl)**

The diagrams showed the interaction and preferred conformation of emodin at the retained myristoyl-binding pocket (A) and the ATP-binding site (B) with labeled amino residues which greatly promoted binding free energy. Emodin was yellow. The hydrogen-bond was indicated by purple dotted line. (Color figure can be accessed in the online version.)
with imatinib is effective against Bcr-Abl overexpression. The binding free energies and the amino acid residues interacted with emodin at the ATP-binding and sites were obtained to evaluate the binding affinity of emodin. The docking study showed that emodin bound to the Abl binding site in the hinge region by weak H-bonds and hydrophobic interaction with binding free energy of $-13.159 \text{ kcal/mol}$, while emodin formed four strong H-bands and hydrophobic bonds at the allosteric site with a less negative binding free energy, which indicated that emodin at the low concentration preferred to be an allosteric inhibitor targeting the myristoyl-binding pocket, instead of Bcr-Abl TKIs. Conversely, at the high dosages of 60 and 80$\mu$M, emodin alone significantly decreased p-Bcr-Abl and Bcr-Abl expression levels, just like the mechanism of Bcr-Abl TKIs (data not shown). Relevant to this study, combining and Bcr-Abl expression levels, just like the mechanism of Bcr-Abl inhibition, an allosteric inhibitor with ATP site TKIs re-inforces target inhibition in Bcr/Abl-positive clinical isolates and cell lines. Although the structural details of Bcr-Abl bound with emodin and imatinib have not been explored, our study suggested that emodin inhibited resistance to imatinib by an allosteric inhibition of Bcr-Abl.

In summary, our study highlighted that emodin, a natural anthraquinone, had effective potency in inhibiting imatinib-resistance caused by Bcr-Abl overexpression in K562/G01 cell line. Emodin significantly sensitized imatinib-induced apoptosis by downregulating Bcr-Abl/STAT5c-MYC/MCL-1 signaling pathway and downstream proteins related with cellular proliferation and apoptosis. Moreover, emodin could bind to the myristoyl-binding pocket with much higher affinity than to the ATP-binding site, which elucidated well the ability of emodin to potentiate the efficacy of imatinib. Our data suggested emodin might be a chemotherapeutic agent that inhibits imatinib-resistance for CML treatment.

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Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

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