Wnt5a enhances the response of CML cells to Imatinib Mesylate through JNK activation and γ-catenin inhibition

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Imatinib Mesylate is widely used for the treatment of chronic myelogenous leukaemia (CML), and its effects on CML cells are influenced by several signalling proteins. The research is aimed at determining whether Wnt5a affects the effects of Imatinib Mesylate against BCR-ABL positive CML cells (K562 cells and KUB12 cells) and which signalling proteins are involved in. The results showed that Wnt5a augmented the effects of Imatinib Mesylate on inhibiting CML cells proliferation and inducing apoptosis in vitro; Wnt5a enhanced the inhibition effect of Imatinib Mesylate on the growth of K562 cells xenograft tumour in an animal model. Furthermore, Wnt5a inhibited β-catenin and its target gene Survivin, increased the activity of JNK and suppressed γ-catenin expression. When inhibiting the activity of JNK, the influence of Wnt5a on the effects of Imatinib Mesylate was attenuated. Moreover, JNK suppressed β-catenin and its target gene Survivin, and enhanced the effects of Imatinib Mesylate. These results suggest that Wnt5a can enhance the efficacy of Imatinib Mesylate through JNK/β-catenin/Survivin and γ-catenin/β-catenin/Survivin pathways.

1. Introduction

BCR-ABL fused protein is characteristic of chronic myelogenous leukaemia (CML). It activates several signalling pathways, such as: PI3K-AKT pathway, Ras-Raf-MEK-ERK pathway and Jak-Stat pathway, and the activation of these pathways leads to unregulated growth and resistance to apoptosis [1–3]. Imatinib Mesylate, a BCR-ABL tyrosine kinase inhibitor, is widely used for treating CML. It has been reported that modulating some signalling optimized the effects of Imatinib Mesylate on CML cells, instance, the inhibition of Fzd8, β-catenin, CaMKII, Ca2+ or Survivin enhanced the effects of Imatinib Mesylate [4–6]. Among them, β-catenin plays important roles in the survival and renewal of CML cells [7], and the suppression of β-catenin [4,5,8,9] or its target gene Survivin [6,10,11] makes CML cells more susceptible to Imatinib Mesylate.

Wnt5a, a member of the Wnt family, plays different roles in different kinds of tumours, which is the result of Wnt5a binding to different receptors and being involved in different signalling pathways [12]. In some cells, Wnt5a binds the Ror2 receptor and subsequently activates JNK [12–14]. JNK was required for the transformation of BCR-ABL-positive acute lymphoblastic leukaemia (ALL) [15,16]. However, in CML, the study on JNK is limited and there are different viewpoints [17]. In the study of Mancini, Imatinib Mesylate increased the activation of JNK, and drove BCR-ABL-expressing cells towards apoptosis [18]. Moreover, potentiating the effects of Imatinib Mesylate with some pharmacological therapies was accompanied by JNK activation [19–21].

In addition, γ-catenin was upregulated by BCR-ABL, and downregulated γ-catenin reduced the growth of CML cells and sensitized them to Imatinib Mesylate, and γ-catenin upregulated β-catenin through GSK3β in CML cells [22].

5-Aza-2′-deoxycytidine, a DNA methyltransferase inhibitor, had a synergic effect with Imatinib Mesylate on K562 cells which are BCR-ABL positive CML cells [23]. Previously, both our and Ying’s studies showed that Wnt5a expression was decreased in K562 cells due to its methylated promoter [24,25]. And 5-aza-2′- deoxycytidine has the ability to recover the expression of Wnt5a [24], so we wondered whether Wnt5a could affect the response of CML cells to Imatinib Mesylate. This study showed that Wnt5a enhanced the anti-leukaemia effect of Imatinib Mesylate, promoted the activity of JNK, suppressed γ-catenin, and inhibited β-catenin and its target gene Survivin. JNK also enhanced the anti-leukaemia effect of Imatinib Mesylate. Therefore, the finding suggests that Wnt5a can enhance the effects of Imatinib Mesylate, and the mechanism is related to that Wnt5a inhibited β-catenin and Survivin through JNK activation and γ-catenin inhibition.
2. Materials and methods

2.1. Cell lines and chemicals

K562 cells, KUB12 cells (BCR-ABL positive CML cells) and HEK293 cells were purchased from Shanghai Cell Bank (Shanghai, China). K562 cells were maintained in RPMI 1640 (HyClone) medium with 10% FBS (Fetal Bovine Serum, Gibco); KUB12 cells were maintained in IMDM (HyClone) medium with 15% FBS; HEK293 cells were maintained in DMEM (HyClone) medium with 10% FBS.

Imatinib Mesylate (Selleck Chemical) and SP600125 (Calbiochem) were dissolved in DMSO (Sigma–Aldrich).

2.2. Plasmids and cDNA

The retroviral vectors pSEB-flag and pCL-ampho were gifts from Dr. Zuo Guowei. JNK1 and JNK2 cDNA were gifts from Professor Gao Ning. The pSEB-Wnt5a plasmids were constructed previously [26]; JNK1 and JNK2 cDNA were cloned into the pSEB-flag plasmid (pSEB-JNK1, pSEB-JNK2).

2.3. Stable cell lines construction

The plasmid (pSEB-Wnt5a, pSEB-JNK1, pSEB-JNK2 or pSEB-flag) was transfected into HEK293 cells with pCL-ampho and Liposome 2000 (Invitrogen), and the supernatants were collected. The K562 cells were infected with the retrovirus were obtained.

K562 cells infected with the retroviruses containing JNK1 cDNA, JNK2 cDNA or an empty vector were referred to as K562-JNK1 cells, K562-JNK2 cells, or K562-vector cells, respectively. KUB12 cells infected with retroviruses containing Wnt5a cDNA, JNK1 cDNA, JNK2 cDNA or an empty vector were referred to as KUB12-Wnt5a cells, KUB12-JNK1 cDNA, KUB12-JNK2 and KUB12-vector cells, respectively.

2.4. Cell proliferation and apoptosis analysis

K562 or KUB12 cells were plated into 96-well plates (1.5 × 10^4 cells/well). After the cells were treated with different doses of Imatinib Mesylate for 24 h, 10 μL CCK-8 reaction (Dojindo, Japan) was added into the plates and then the plates were incubated at 37 °C for 90 min. The absorbance values were then monitored with Bio-Rad Microplate Reader (USA).

An apoptosis analysis assay was carried out according to the manufacturer’s instruction (Keygenent, China). K562 or KUB12 cells were plated into the 6-well plate (5 × 10^4 cells/well). The cells, after being treated with Imatinib Mesylate (K562, 5 μmol/L, KUB12, 0.25 μmol/L) for 18 h, were washed with PBS for three times. They were then re-suspended in 500 μL of binding buffer, and 5 μL of Annexin V-APC and 2 μL of PI (propidium iodide, Sigma–Aldrich) were added. Finally, the cells were analysed using Flow Cytometry (BD Biosciences, USA). The cells with Annexin V-APC positive and PI negative were regarded as apoptosis cells.

2.5. Western blotting

Western blotting was carried out as described previously [27]. The following antibodies were used: Wnt5a, phospho-JNK (Tyr177), β-catenin, phospho-β-catenin (Ser33/37/Thr41) and Survivin antibodies were purchased from Cell Signalling; γ-catenin antibody was purchased from Abcam; And β-actin antibody was purchased from Santa Cruz.

2.6. In vivo experiment

The NOD/SCID mice (4 weeks old, female) were purchased from the Experimental Animal Centre (Third Military Medical University, Chongqing, China) and were bred in the SPF animals facilities. Sixteen mice were divided into four groups at random and were injected subcutaneously with 1.5 × 10^7 K562-vector cells or K562-Wnt5a cells (constructed previously [26]) into the right axilla. Four mice injected with the K562-vector cells and four mice injected with the K562-Wnt5a cells were also injected intraperitoneally with Imatinib Mesylate (40 mg/kg, diluted in PBS) on the 6th, 9th, 12th, 15th, and 18th days, and the remaining mice were injected with the solvent control. The tumour volume was calculated as follows: V = 1/2 × L × W^2 (L=length, W=width). After the mice were sacrificed, the tumours were excised. The experiments were performed in accordance with the guidelines set by the Ethical Committee of Third Military Medical University, and were approved by that the Ethical Committee of Third Military Medical University (approval number: 201002005).

2.7. Statistical analysis

An unpaired Student’s t-test was used to determine statistical significance with SPSS software (13.0). p<0.05 was considered significant.

3. Results

3.1. Wnt5a over-expression enhanced the effects of Imatinib Mesylate on CML cells

Previously, we constructed the K562 cells over-expressing Wnt5a (K562-Wnt5a) [26]. In this study, we constructed KUB12 cells over-expressing Wnt5a (KUB12-Wnt5a) as described in Section 2 (Fig. 1A).

After exposure to different doses of Imatinib Mesylate for 24 h, the proliferations of Wnt5a-overexpression cells were more decreased than those of the control cells (Fig. 1B and C). After treating the cells with Imatinib Mesylate for 18 h (K562, 5 μmol/L; KUB12, 0.25 μmol/L), the apoptosis percentages of Wnt5a-overexpression cells were higher than those of the control cells (Fig. 1D and E), and the levels of BCR-ABL and phosphor-BCR-ABL were lower in the Wnt5a-overexpression cells than in control cells (Fig. 1F).

3.2. Wnt5a suppressed γ-catenin, β-catenin and enhanced the activity of JNK in CML cells

To investigate the signalling events associated with Wnt5a affecting Imatinib Mesylate in CML cell, we detected the effects of Wnt5a on γ-catenin, β-catenin and JNK. Our results showed that the expression levels of γ-catenin were lower in Wnt5a-overexpressing CML cells than in control cells (Fig. 1G). Previous study showed that, Wnt5a could not change β-catenin expression in K562 cells, but it could up-regulate the level of phosphor-β-catenin [27], thereby promoting the degradation of β-catenin. Here, we showed that β-catenin expression was lower and the level of phosphor-β-catenin was higher in KUB12-Wnt5a cells than in the control cells; (Fig. 1G). Survivin was considered as one of target genes of β-catenin [11], and our study showed that the expression of Survivin were suppressed in Wnt5a-overexpression cells compared to the control cells (Fig. 1G). The result also showed that Wnt5a did not affect the expression of JNK but increased the levels of phosphor-JNK (Fig. 1G).

3.3. The JNK inhibitor, SP600125, abrogated the Wnt5a-induced elevated effects of Imatinib Mesylate on CML cells

To demonstrate whether JNK was involved in Wnt5a enhancing the effects of Imatinib Mesylate, the activity of JNK was inhibited with SP600125 (30 μmol/L), then K562-Wnt5a and KUB12-Wnt5a cells were used to analyse the effects of Wnt5a on Imatinib Mesylate. After Imatinib Mesylate treating for 24 h, the proliferations of the cells pre-treated with SP600125 were higher than the untreated control cells (Fig. 2A–C); Imatinib Mesylate also induced less apoptotic cells in the cells pre-treated with SP600125 than in the controls (Fig. 2D and E).

3.4. JNK inhibited β-catenin in CML cells

To further study the role of JNK in the effects of Imatinib Mesylate, we constructed JNK1 and JNK2 overexpressing CML cells (K562-JNK1, K562-JNK2, KUB12-JNK1 and KUB12-JNK2 cells). The expressions of β-catenin in K562-JNK1, K562-JNK2 cells were not obviously different from the control cells, but the levels of phosphor-β-catenin were higher than those in the control cells. In KUB12-JNK1 and KUB12-JNK2 cells, β-catenin expressions were lower than that in the control cells, and the levels of phosphor-β-catenin were higher than those in the control cells (Fig. 3A). JNK1 or JNK2 suppressed the expression of Survivin but had no obvious influence on γ-catenin.
3.5. JNK enhanced the effects of Imatinib Mesylate on CML cells

We also tested the influences of JNK on the effects of Imatinib Mesylate. Enhanced effects of Imatinib Mesylate by JNK were also observed: The decreased proliferation of JNK1 and JNK2 overexpression cells suppressed by Imatinib Mesylate were more significantly than the control cells (Fig. 3B and C); The percentages of apoptotic JNK1 and JNK2 overexpression cells induced by Imatinib Mesylate were higher than that of the control cells (Fig. 3D and E); BCR-ABL and phosphor-BCR-ABL in these cells were lower compared to control cells upon exposure to Imatinib Mesylate (Fig. 3F).
3.6. Wnt5a enhanced the inhibition effects of Imatinib Mesylate on K562 cells xenograft tumour in an animal model

Finally, we investigated the influences of Wnt5a on the effects of Imatinib Mesylate on CML cells in NOD/SCID mice. After treated K562-Wnt5a xenograft tumours with Imatinib Mesylate, from day 6 to 21 the tumour size of K562-Wnt5a cells yielded a 1.7-fold increase, which was less dramatic than that of the control cells (approximately 4.8-fold increase) (Fig. 4A and B).

4. Discussion

Previously, our study showed that Wnt5a bounded Ror2 receptor and inhibited canonical Wnt signalling in K562 cells [27]. The reports suggest Wnt5a acts as a tumour inhibitor in CML [24–26], and CML spontaneously arisen in Wnt5a-deficient mice [28]. In the present report Wnt5a enhanced proliferation inhibition and apoptosis induction effects of Imatinib Mesylate on CML cells, which is consistent with these earlier reports, and the roles of Wnt5a were related to suppressing β-catenin and Survivin through JNK activation and γ-catenin inhibition (Fig. 4C). In addition, because of the ability of 5-aza-2′-deoxycytidine to recover the expression of Wnt5a in K562 cells [24], our study may provide an explanation for that 5-aza-2′-deoxycytidine had a synergic effect with Imatinib Mesylate on K562 cells [23].

It has been shown that the downregulation of β-catenin synergized with Imatinib Mesylate in inhibiting CML cells [4,5], and Imatinib Mesylate combined with inhibiting β-catenin by genetic inactivation or pharmacologic modulation was shown more effective for treating BCR-ABL-induced CML in a mouse model [9]. Survivin, one of target genes of β-catenin [11], acts as an inhibitor of apoptosis [29], and it is regarded as a therapy target for a serias of tumours [29], besides, inhibition of Survivin makes tumour cells more susceptive to anticancer drugs [30]. In CML, higher levels of Survivin were observed in accelerated phase and blastic phase patients compared with chronic phase patients [31,32], and it has been reported disruption of Survivin promoted the apoptosis of the BCR-ABL positive cells induced by Imatinib Mesylate [6,10]. Our previous report demonstrated that Wnt5a blocked the function of β-catenin by promoting its phosphorylation in K562 cells [27]. Here, we also confirmed that Wnt5a blocked β-catenin in the KU812 cells and blocked Survivin expression in both the K562 cells and KU812 cells. So we believe that inhibition of β-catenin and Survivin is the mechanism by which Wnt5a enhanced the effects of Imatinib Mesylate. However, the study of Gregory et al. [4] identified that Wnt5a may be a resistance factor in K562 cells through Synthetic Lethal Screen, but they did not further confirm the roles of Wnt5a in the effects of Imatinib Mesylate. Nevertheless, we showed that Wnt5a enhanced the effects of Imatinib Mesylate not only in K562 cells but also in KU812 cells, and we also confirmed that Wnt5a enhanced the inhibition effect of Imatinib Mesylate on K562 xenograft tumours of NOD/SCID mice.

Furthermore, our study showed that Wnt5a promoted the activity of JNK, and inhibition of JNK with SP600125 attenuated the enhanced effects by Wnt5a. Hu et al. [33,34] demonstrated that β-catenin expression was increased in JNK1- or JNK2-deficient mice and JNK can inhibit β-catenin expression, and Lee et al. [35] showed that JNK promoted β-catenin phosphorylation. Liao et al. [36] also demonstrated that JNK blocks nuclear β-catenin accumulation in Xenopus embryos. However, other papers [37,38] demonstrated that JNK and β-catenin form complexes that promote β-catenin translocation into the nucleus to function as a transcription factor. Additionally, studies on the function of JNK in CML cells were limited and in disagreement [17]. Our results have demonstrated that both JNK1 and JNK2 can inhibit β-catenin as well as its downstream genes Survivin, which led to promoting the sensitivity.
of CML cells to Imatinib Mesylate. This observation is in agreement with the roles of JNK in CML cells in previous studies [19–21]. Furthermore, we found that the roles of JNK may be different in CML and BCR-ABL-positive ALL cells, which may be attributable to the different lineage (myeloid and lymphoid) of BCR-ABL-positive leukemia cells.

Some Wnt proteins have been reported to regulate γ-catenin. For example, Wnt1 increases the expression of γ-catenin in PC12 cells [39], and Wnt3a increases the expression of γ-catenin in NCI-H28 cells [40]. In this study, we showed Wnt5a suppressed the expression of γ-catenin. Our previous study showed that down-regulated γ-catenin reduced the growth of CML cells and sensitized them to Imatinib Mesylate through inhibiting β-catenin and Survivin [22], so inhibiting γ-catenin/β-catenin/Survivin is another mechanism for the roles of Wnt5a in CML.

This report shows that Wnt5a enhances the effects of Imatinib Mesylate on CML cells through JNK activation and γ-catenin inhibition. Both JNK activation and γ-catenin inhibition lead to inhibition of β-catenin and Survivin. We expect this study can further the understanding of the Wnt5a-related pathways as well as provide a new strategy for CML treatment.

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Conflict of interest

The authors were informed consent and declared there was no conflict of interest.

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References


