

Review Article

Ginsenoside Rg1 as a Potential Regulator of Hematopoietic Stem/Progenitor Cells

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Ginsenoside Rg1 (Rg1), a purified, active component of the root or stem of ginseng, exerts positive effects on mesenchymal stem cells (MSCs). Many recent studies have found that hematopoietic stem cells (HSCs), which can develop into hematopoietic progenitor cells (HPCs) and mature blood cells, are another class of heterogeneous adult stem cells that can be regulated by Rg1. Rg1 can affect HSC proliferation and migration, regulate HSC/HPC differentiation, and alleviate HSC aging, and these findings potentially provide new strategies to improve the HSC homing rate in HSC transplantation and for the treatment of graft-versus-host disease (GVHD) or other HSC/HPC dysplasia-induced diseases. In this review, we used bioinformatics methods, molecular docking verification, and a literature review to systematically explore the possible molecular pharmacological activities of Rg1 through which it regulates HSCs/HPCs.

1. Introduction

Ginsenosides are the active components of ginseng and comprise a group of sterol compounds. According to differences in their glycosidic structure, ginsenosides are divided into two subtypes: the dammarane type and the oleanane type [1, 2]. Ginsenoside Rg1 (Rg1, molecular formula: $C_{42}H_{72}O_{14}$, Figure 1(a), image from PubChem), a member of the ginsentriol subtype of dammarane ginsenosides, is an important monomeric ginsenoside and the most abundant component of Chinese/Korean ginseng. Rg1 not only acts on the nervous, cardiovascular, blood, and immune systems but also exhibits a variety of positive pharmacological activities, such as its neuroprotective activity [3] and its abilities to treat myocardial ischemia [4] and repair hematopoietic immune disorders [5]. To date, several clinical trials on the use of drugs containing Rg1 for the treatment of vascular dementia, hyperlipidemia, hypertension, Sjögren's syndrome, rheumatic diseases, and ischemic stroke have been registered on clinicaltrials.gov [6, 7].

Hematopoietic stem cells (HSCs) are adult stem cells that can self-renew, differentiate into blood cell lineages, and exert long-term effects on maintaining and producing all mature blood cell lineages during the life cycle of an organism [8]. Under a stable metabolism status, most HSCs are in a static state (quiescent HSCs), whereas hematopoietic progenitor cells (HPCs) actively proliferate and maintain the daily hematopoietic function. When the body is stimulated, such as during life-threatening blood loss, infection, and inflammation, HSCs can be activated in the bone marrow to proliferate and participate in blood formation [9, 10]. HSCs mainly function in the specific bone marrow microenvironment (HSC niche), which provides the signals needed to protect HSCs and maintain HSC differentiation [11]. Although the surface phenotypes of human and murine HSCs differ, these cell types possess the basic functions of HSCs. CD34 is a marker of human HSCs, and clinical transplantation studies using enriched CD34⁺ bone marrow cells have indicated the presence of HSCs with the ability to reconstitute bone marrow within this fraction [12, 13]. For differentiation and functional

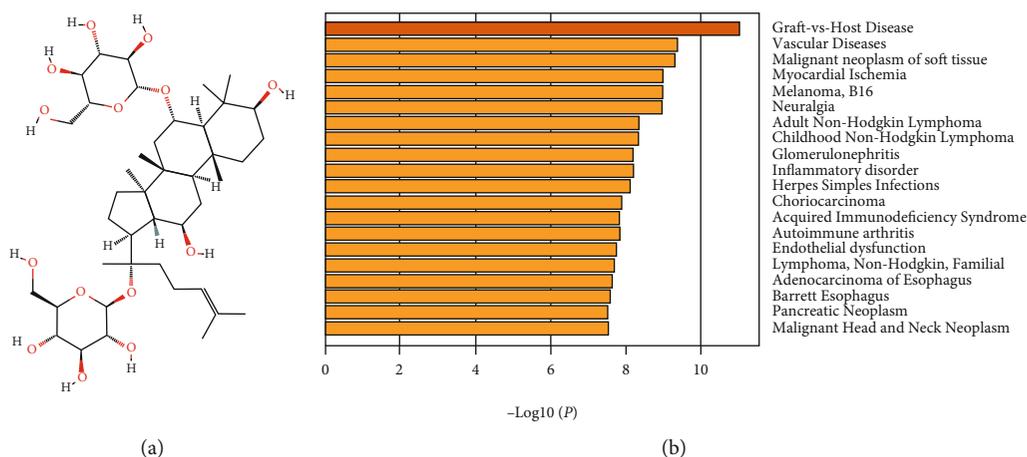


FIGURE 1: Molecular structure of Rg1 and the diseases that may be targeted by Rg1. (a) Molecular structure of ginsenoside Rg1. (b) GVHD is the top putative disease targeted by Rg1, as shown by an analysis using the Metascape platform.

research involving murine HSCs, a variety of markers are commonly used to identify or isolate HSCs. In general, murine HSCs do not express lineage-specific markers (Lin^{-}) and are positive for c-kit (CD117⁺ or c-Kit⁺) and stem cell antigen-1 (Sca-1⁺). More recently, the HSC population was further clarified to include the markers CD150 and CD48, and as a result, this population was defined by the marker profile $Lin^{-}c\text{-Kit}^{+}Sca\text{-1}^{+}CD48^{-}CD150^{+}$ [14–16].

In a previous study, we described the active regulatory effects of Rg1 on the proliferation, differentiation, senescence, and apoptosis of mesenchymal stem cells (MSCs) [17]. Here, HSCs, a heterogeneous adult stem cell population that completely differs from MSCs, were analyzed and systematically reviewed. We used bioinformatics methods to analyze the potential molecular pharmacological role of Rg1 in HSC/HPC regulation and reviewed the literature to summarize the mechanisms through which Rg1 activates HSC proliferation and differentiation and its antiaging effects in HSCs/HPCs.

2. Prediction of Potential Rg1 Targets in HSCs/HPCs Based on a Bioinformatics Analysis

The molecular structure of Rg1 from PubChem was analyzed to identify putative targets of Rg1, and the TargetNet and SwissTargetPrediction platforms were also used to predict putative targets of the ginsenoside Rg1 [18, 19]. A comprehensive search identified 723 putative targets (623 from TargetNet and 100 from SwissTargetPrediction) of Rg1 (Supplementary Materials 1 and 2). Furthermore, the top 20 targets (10 from TargetNet and 10 from SwissTargetPrediction) with relatively high probability among the putative targets were pooled and used to predict associated diseases that may be regulated by Rg1 (analyzed by DisGeNET using the Metascape platform [20]). The results showed that graft-versus-host disease (GVHD) is the top putative targeted disease that may be regulated by Rg1 (Figure 1(b)). This finding suggests that Rg1 could be used as a potential monomeric drug to reduce GVHD and that Rg1 could further improve the success rate of HSC transplantation.

To perform an interactive bioinformatics analysis of the relationships between HSC proliferation and migration and Rg1, we analyzed the molecular functions of Rg1 through a metaenrichment of pathway. Genes that may be related to HSC proliferation or migration were analyzed using the Comparative Toxicogenomics Database, and we identified 49 genes associated with HSC proliferation and 23 genes (after removing repetition) associated with HSC migration (Supplementary Materials 3 and 4). A metaenrichment analysis of Rg1 and HSC targets was performed using the Metascape platform, and the results showed that G protein-coupled receptor binding was enriched in the effects of Rg1 on HSC proliferation, whereas integrin binding and protein homodimerization activity were enriched in the effects of Rg1 on HSC migration (Figure 2).

In addition, we used bioinformatics methods to analyze the potential molecular biological functions of Rg1 during the process of HSC or HPC differentiation. Putative targets of HSC or HPC differentiation were obtained using the Comparative Toxicogenomics Database. A total of 183 and 359 genes (after removing repetition) related to HSC and HPC differentiation, respectively, were identified (Supplementary Materials 5 and 6). The results of the metaenrichment analysis of pathways using the Metascape platform showed that transcription factor binding and endopeptidase activity are enriched molecular functions in HSC differentiation that may be regulated by Rg1. Moreover, protein domain-specific binding was found to be an additional molecular function that may be regulated by Rg1 during HPC differentiation (Figure 3).

Interestingly, the use of JVenn to visualize the specific targets through which Rg1 regulates HSCs [21] revealed that angiotensin-converting enzyme (ACE, GeneID: 1636) was the only overlapping gene through which Rg1 regulates HSC proliferation and HSC/HPC differentiation (Figure 4). We then used molecular docking to verify the interaction between Rg1 and ACE. Briefly, the crystal structures of putative targets were obtained from the Protein Data Bank, and AutoDock Tools 1.5.6-Vina software was used for the analysis of binding ability and sites. Additionally, PyMOL was utilized to visualize

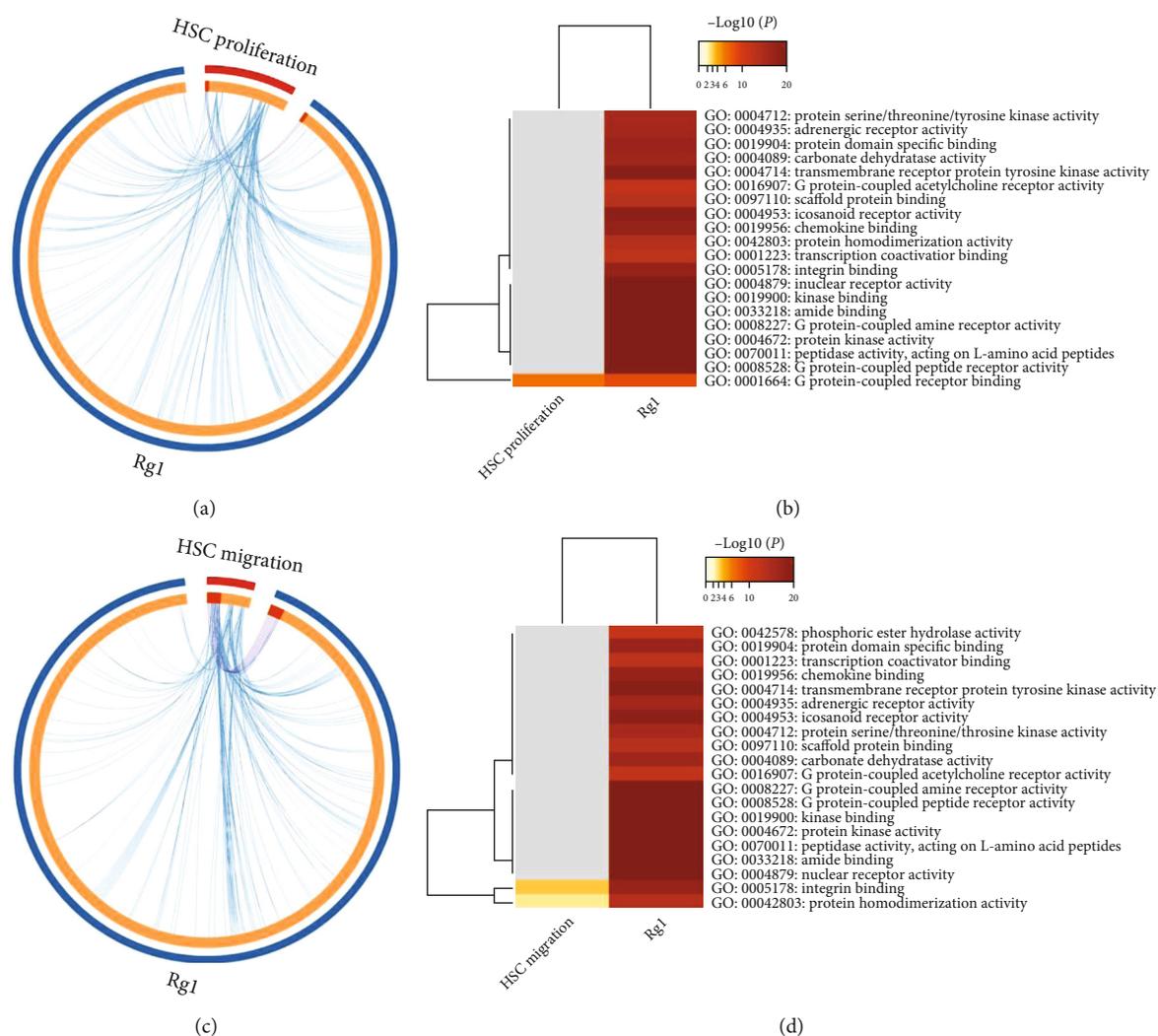


FIGURE 2: Metaenrichment analysis of the molecular effects of Rg1 on HSC proliferation or migration. (a) Ring summary shows overlapping genes related to Rg1 and HSC proliferation at the gene level (the purple line shows the overlapping genes; the blue line shows a functional correlation between genes). (b) Heatmap of terms enriched in the list of genes targeted by Rg1 to regulate HSCs. The terms are colored based on the p value. (c) Ring summary of overlapping genes through which Rg1 regulates HSC migration at the genetic level. (d) Heatmap of terms enriched in the genes targeted by Rg1 to regulate HSC migration. The terms are colored based on the p value.

the interaction between Rg1 and the ACE peptide chain. The results showed that several hydrogen bonds may form between Rg1 and ACE. Specifically, the use of the minimum binding energy (affinity: -13.0 kcal/mol) in the docking analysis revealed that hydrogen bond formed between Rg1 and threonine in chain A of ACE (Figure 4).

Furthermore, the signal pathways and molecular mechanism in the bioinformatics results provide some novel research directions and may be worth further exploration in *in vitro/in vivo* experiments. All the databases used in the study are listed in Supplementary Material 7.

3. A Review of the Literature Reveals That Rg1 Regulates HSC Proliferation, Differentiation, and Migration

HSCs differentiate into myeloid progenitor cells and prolymphoid progenitor cells in the bone marrow to drive bone

marrow hematopoiesis [11, 22]. A previous study found that Rg1 can regulate calcium-sensing receptor (CaSR) to increase the number of $\text{Lin}^- \text{Sca-1}^+ \text{c-Kit}^+$ HSCs and lymphoid CD3^+ cells in the bone marrow and peripheral blood of CY-induced myelosuppressed mice and thereby restores bone marrow function [23]. Interestingly, an analysis of the 9 overlapping target genes through which Rg1 regulates HSC differentiation using the Metascape platform revealed that the calcium signaling pathway was the key KEGG pathway through which Rg1 regulates HSC differentiation (Figure 5(a)). This consistent finding confirms that the CaSR-mediated calcium signaling pathway may be a crucial target through which Rg1 regulates HSC differentiation.

In addition, some studies have shown that Rg1 improves the hematopoietic activity of the bone marrow through extramedullary hematopoiesis. Cyclophosphamide (CY) can cause bone marrow cytotoxicity, leading to bone marrow suppression and triggering extramedullary

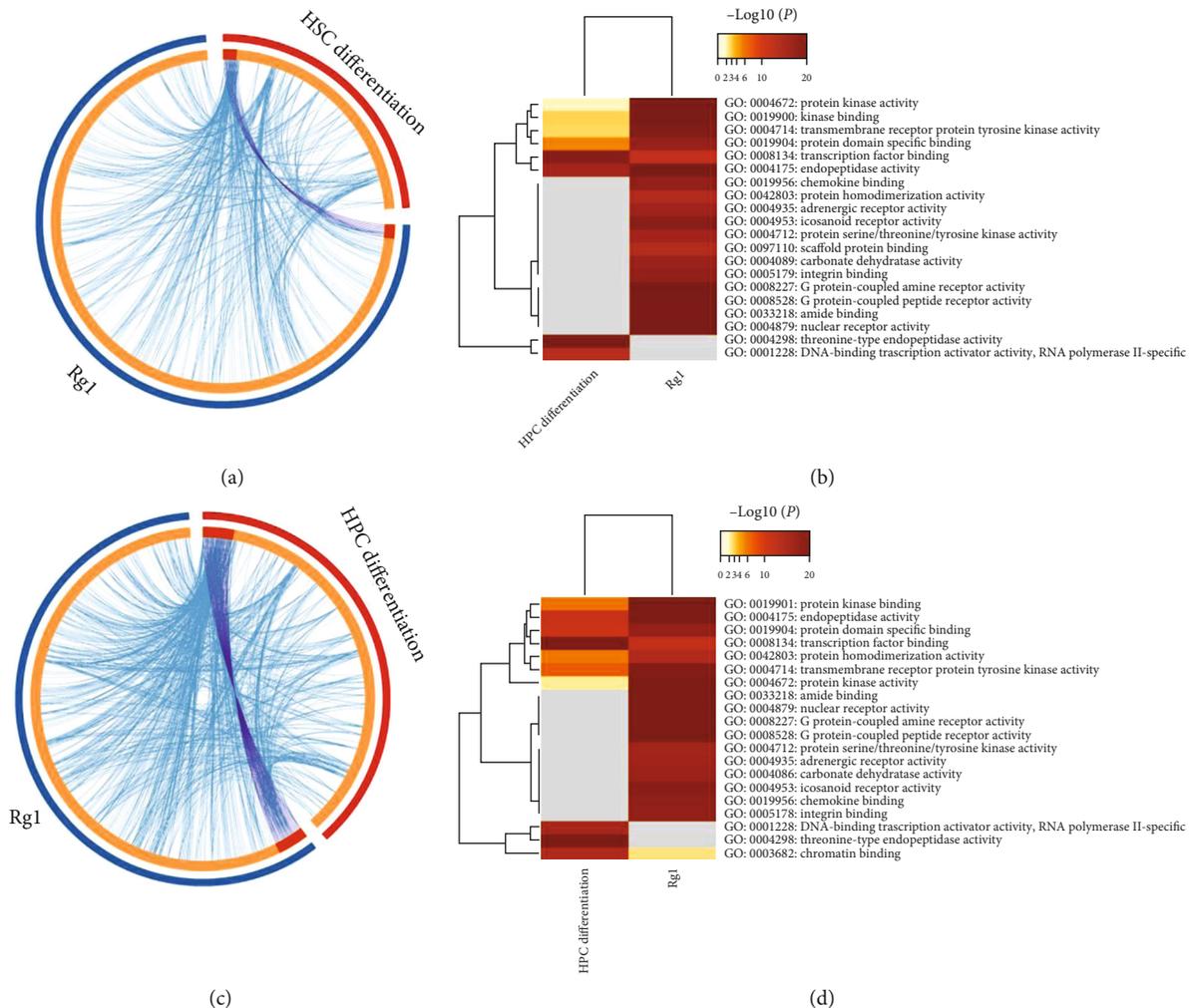


FIGURE 3: Metaenrichment analysis of the molecular effects of Rg1 on HSC/HPC differentiation. (a) Ring summary of overlapping genes through which Rg1 regulates HSC differentiation at the genetic level (the purple line shows overlapping genes; the blue line shows a functional correlation between genes). (b) Heatmap of terms enriched in the genes targeted by Rg1 to regulate differentiation. The terms are colored based on the p value. (c) Ring summary of overlapping genes through which Rg1 regulates HPC differentiation at the genetic level. (d) Heatmap of terms enriched in the genes targeted by Rg1 to regulate HPCs differentiation. The terms are colored based on the p value.

hematopoiesis [24, 25]. Extramedullary hematopoiesis is characterized by the presence of pluripotent HPCs, including erythroid lineage cells, myeloid lineage cells, and megakaryocytes, in the spleen and liver [26]. Liu et al. [27] found that Rg1 treatment could effectively reduce the weight of the spleen of CY-stimulated mice and reduce the absolute number of c-Kit⁺ HSCs in the spleen and that these effects are not caused by apoptosis, which suggests that Rg1 alleviates CY-induced extramedullary hematopoiesis in the spleen. Further research shows that Rg1 could upregulate the proliferative activity of c-Kit⁺ HSCs in the spleen but not in the bone marrow of CY-stimulated mice. Moreover, Rg1 increases the number of c-Kit⁺/CD45⁺ HSCs in the peripheral circulatory system. Most importantly, the effect of Rg1 on HSCs in the bone marrow and peripheral blood is not observed in splenectomy- and CY-induced mice. These results systematically indicate that Rg1 improves CY-induced myelosuppression by activating HSC prolifera-

tion in the spleen, particularly by allowing the homing of HSCs from the spleen through the circulatory system to the bone marrow [27]. In addition, the selective regulation of HSCs in the spleen but not in the bone marrow also suggested that the "spleen-bone marrow" axis homing of HSCs plays a main/crucial role in Rg1 relieving extramedullary hematopoiesis and myelosuppression. Moreover, quiescent HSCs in bone marrow HSC "niche" can maintain hematopoietic homeostasis [28]. In the above study, after the new "niche" formed, the homing HSCs derived from the spleen may also serve as quiescent HSCs and further benefit bone marrow hematopoietic homeostasis.

Interestingly, whether Rg1 directly activates and promotes the proliferation of quiescent HSCs in the bone marrow niche was an open question. First, CD34⁺ cells account for only 1.5% of human bone marrow mononuclear cells, and murine Lin⁻Sca-1⁺c-Kit⁺ HSCs account for less than 1% of bone marrow cells. The treatment of mice with CY

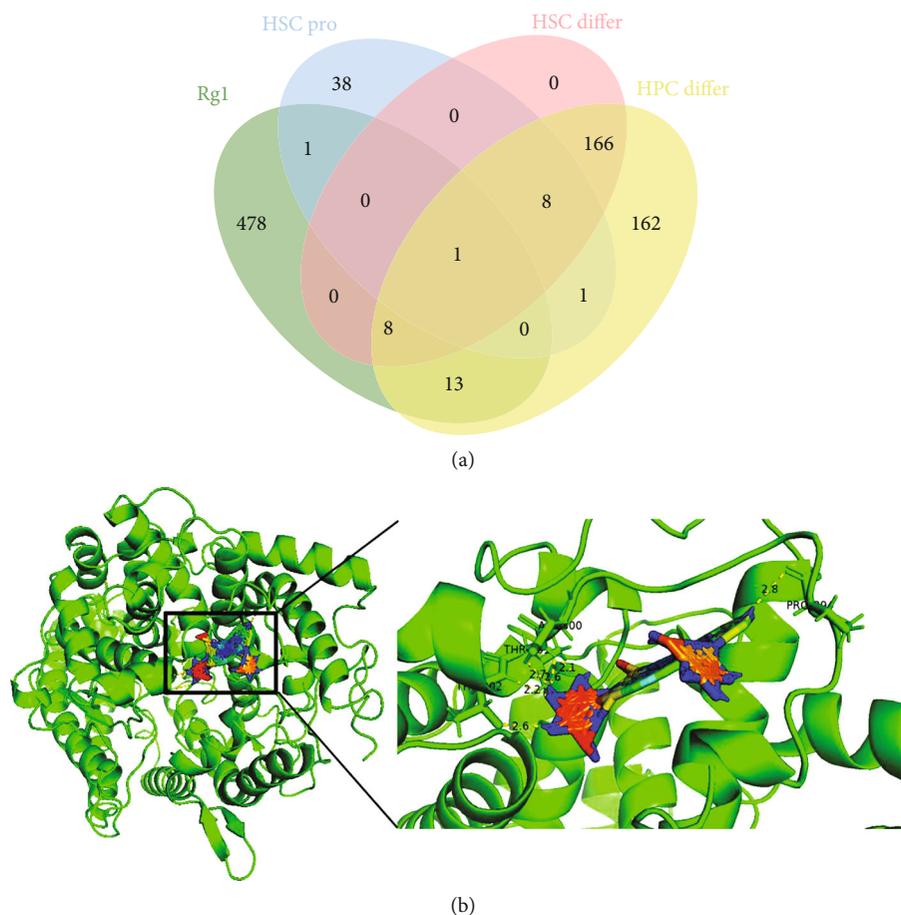


FIGURE 4: Common proteins among putative proteins targeted by Rg1 and proteins related to HSC proliferation, HSC differentiation, and HPC differentiation. (a) Venn diagram showing that one protein (ACE) was found in all four lists. (b) Three-dimensional schematic representation showing the molecular docking model, active sites, and binding distances for Rg1 and ACE after the application of ray tracing.

results in the appearance of a large amount of vacuole-like degradation in the bone marrow cavity and a sharp decrease in the number of bone marrow cells (including MSCs) [23, 29]. In this context, very few HSCs remain in the bone marrow, and the effect of Rg1 on enhancing the mobilization of extramedullary hematopoiesis far exceeds the effect of Rg1 on the mobilization of HSCs in the bone marrow. Therefore, the direct effect of Rg1 on quiescent HSCs in the bone marrow may be difficult to observe. Second, the researchers continuously administered Rg1 (15 mg/kg/day) to CY-induced myelosuppressed mice (splenectomy) for 7 days [27], and the results showed that Rg1 could not effectively increase the percentage of bone marrow $\text{Lin}^- \text{Sca-1}^+ \text{c-Kit}^+$ HSCs (no significant difference), but an increasing trend was observed. Third, Rg1 could also regulate MSCs to protect HSCs from D-galactose- (D-gal-) induced damage [30], and the continuous administration of Rg1 for 7 days could not completely restore the histological morphology of the murine bone marrow (vacuolar pathological structures remained in the bone marrow cavity) [23]. Thus, we infer that the regulatory effect of MSCs on the hematopoietic microenvironment could be delayed. Therefore, further investigation of whether Rg1 can activate HSCs in the bone marrow by prolonging the

duration of Rg1 administration to explore the recovery of bone marrow HSCs in mice with splenectomy is warranted (Figure 5(b)).

In addition, stromal-derived factor-1 (SDF-1)/C-X-C chemokine receptor type 4 (CXCR4) is an important signaling molecule in HSC homing to the bone marrow and bone marrow implantation [31]. Rg1 can regulate the SDF-1 α /CXCR4 axis and plays a regulatory role in the vascular intima [32]. These findings also suggest that Rg1 promotes HSC homing from the spleen to the bone marrow cavity and exerts hematopoietic effects in the bone marrow.

4. Mechanisms Involved in the Attenuation of HSC Aging by Rg1

Traditionally, aging HSCs gradually lose the potential for self-renewal and differentiation, and the likelihood of abnormal metabolic cellular functions greatly increases [33]. An increasing number of studies have shown that inflammation and chemical or physical factors also cause DNA damage, which can lead to HSC aging [10, 34, 35]. Excessive D-gal results in the production of aldohexose and hydrogen peroxide via galactose oxidase and promotes the generation of

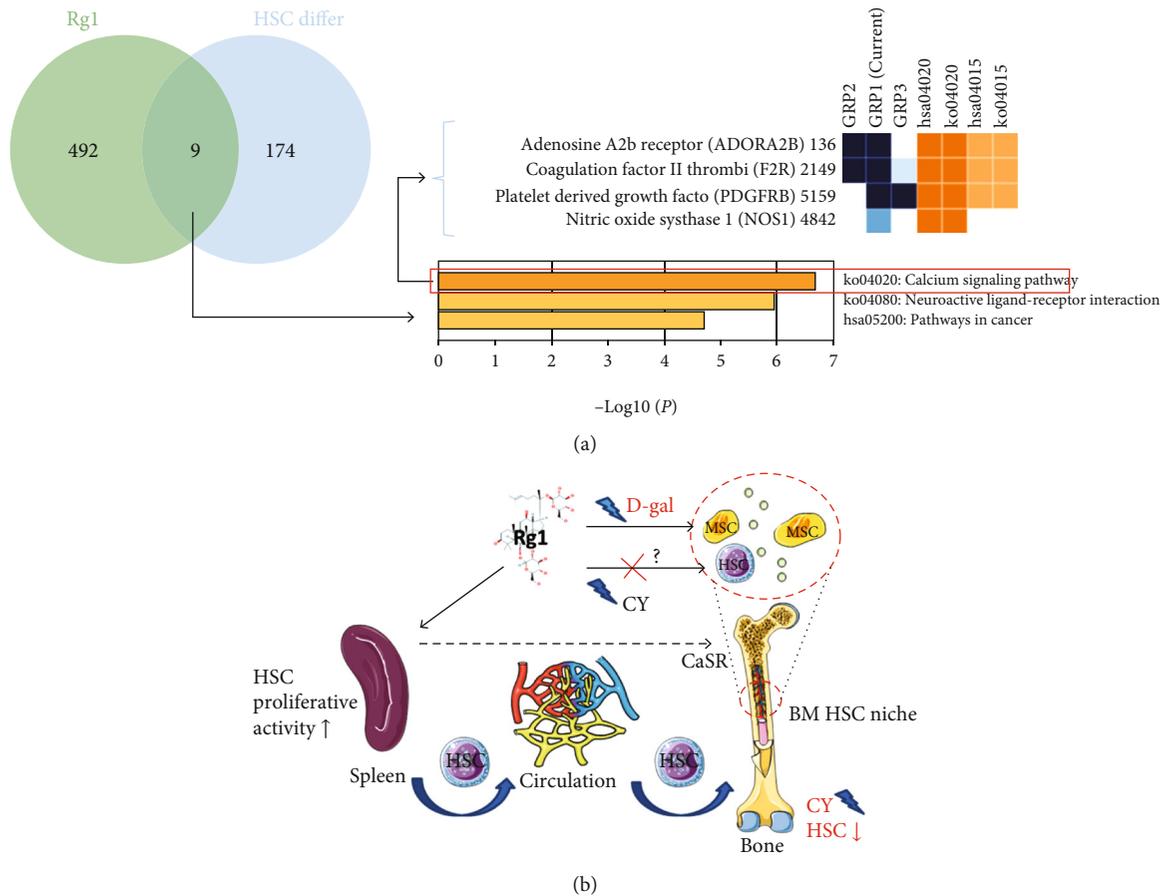


FIGURE 5: Relevant bioinformatics data and mechanistic summary of the mechanism through which Rg1 regulates the proliferation, differentiation, and migration of HSCs. (a) Venn diagram and KEGG pathway enrichment analyses revealed that the calcium signaling pathway is the key pathway through which Rg1 regulates HSC differentiation. (b) Rg1 promotes the homing of HSCs from the spleen to the bone marrow through the peripheral circulatory system in mice with myelosuppression caused by cyclophosphamide. CY: cyclophosphamide; HSC: hematopoietic stem cell; D-gal: D-galactose; CaSR: calcium-sensing receptor; MSC: mesenchymal stem cell.

oxygen-derived free radicals and superoxide anions, which results in impairment of the functions of macromolecules and cells [36, 37]. Rg1 inhibits oxidative stress and reduces DNA damage, which results in enhancement of the antiaging ability of Sca-1⁺HSCs/HPCs in a murine model of D-gal-induced aging, and the effect is related to inhibition of excessive activation of the Wnt/ β -catenin signaling pathway. The classic Wnt/ β -catenin pathway is essential for the regulation of stem cell pluripotency and the determination of cell fate [38, 39]. When D-gal activates the Wnt/ β -catenin pathway, the Wnt ligand (a secreted glycoprotein that binds to Frizzled receptors) forms a large cell surface complex with low-density lipoprotein receptor-related protein (LRP) 5/6. A previous study found that Rg1 can inhibit D-gal-induced overactivation of the Wnt/ β -catenin signaling pathway. Rg1 can reduce β -catenin expression and glycogen synthase kinase 3 beta (GSK3 β) phosphorylation in the cytoplasm and can further reduce the protein expression of β -catenin in the nucleus via Ras-related C3 botulinum toxin substrate 1 (Rac1) and other factors; in addition, the binding of β -catenin to the transcription factor TCF-4 in the nucleus is reduced and ultimately inhibits c-Myc gene expression,

which results in the reduction of β -galactosidase expression [40] (Figure 6).

A previous study also showed that Rg1 could mediate the p53-p21-Rb signaling pathway to improve routine blood index abnormalities caused by lead acetate and alleviate lead acetate-induced HSC aging and aging-related inflammatory responses. Lead acetate can cause DNA damage in HSCs and induce cells to produce γ -H2AX. Rg1 can reduce the DNA damage-induced increases in p53 transcription and translation but does not affect the activity of P16, which results in the amelioration of lead acetate-induced HSC damage [41]. Studies also found that Rg1 can attenuate ROS production to improve HSC function in various settings [40, 42–44]. For example, Rg1 can decrease ROS production and further increase the ratio of Bcl-2/Bax in the radiation-induced HSC mitochondrial apoptosis (Figure 6).

Furthermore, Rg1 may inhibit some key genes in the p16^{INK4a}-Rb, p53-p21^{Cip/Waf1}, and SIRT6/NF- κ B signaling pathways to protect against HSC aging induced by D-gal, t-BHP, and radiation. The mechanism involves reducing DNA damage, regulating the cell cycle, adjusting telomerase activity, and compensating for the HSC telomere length [42, 45–47].

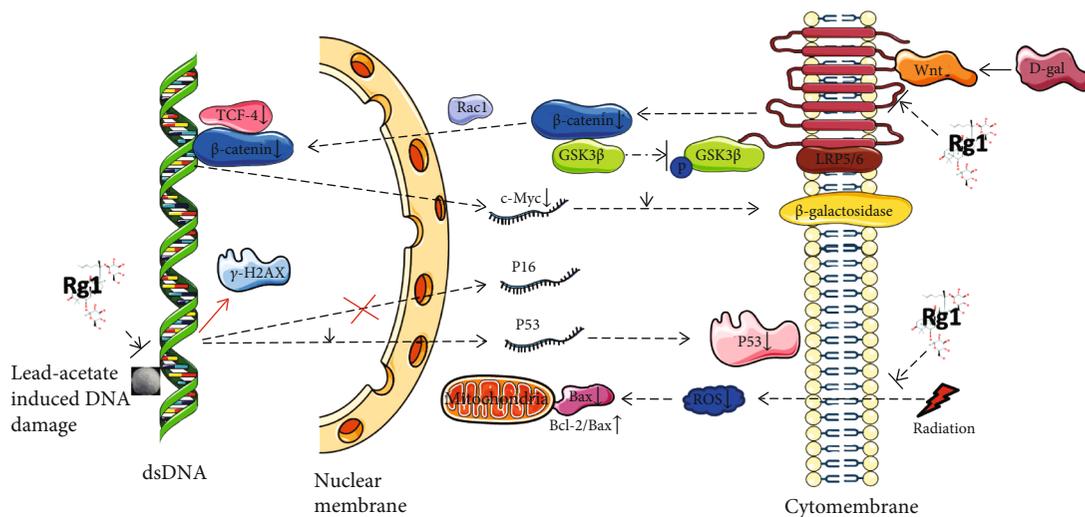


FIGURE 6: Rg1 alleviates HSC aging by regulating the Wnt/ β -catenin and p53 signaling pathways. D-gal: D-galactose; LRP: low-density lipoprotein receptor-related protein; GSK-3 β : glycogen synthase kinase 3 beta; Rac1: Ras-related C3 botulinum toxin substrate 1; TCF-1: transcription factor 4.

5. Overview of the Regulation of HPCs and Mature Blood Cells by Rg1

After HSCs differentiate into multipotent progenitors (MPPs), they develop into common myeloid progenitors (CMPs) and common lymphoid progenitors (CLPs), the classic pathway for the differentiation of HPCs [48].

Ginseng extract affects immune cell functions differently according to the specific ginsenoside profile, and the immunomodulatory effects of various ginsenoside monomers are different [49]. For example, Rg1 inhibits TNF- α expression in THP-1 human leukemia cells, whereas the ginsenoside Rh1 increases TNF- α expression [50]. An Rg1/Rb1 mixture and Rg1 exert different effects on IL-6 and TNF- α [49, 51]. These effects are also exhibited by the effect of total ginsenosides or Rg1 monomers on dendritic cells (DCs). Total saponins in ginseng roots can inhibit the maturation of DCs in the presence of lipopolysaccharide (LPS) [51]. However, 10 μ g/ml Rg1 can increase CD83, CD80, and HLA-DR expression, reduce CD14 expression in DCs derived from human peripheral blood mononuclear cells, and induce DCs to secrete cytokines (IL-6, TNF- α , and IL-1 β) and chemokines (such as IL-8 and IP-10) [52]. Rg1 can stimulate the proliferation of human granulocyte-macrophage progenitors (GMPs) [53]. GMPs can develop into monocytes and myeloblasts. In LPS-activated macrophages, 10 μ M Rg1 can also increase the TNF- α levels and decrease the IL-6 protein levels, and these effects are related to regulation of the NF- κ B and PI3K/Akt/mTOR pathways [54]. Moreover, 50 μ M Rg1 can inhibit RAW264.7 macrophage apoptosis induced by serum deprivation by activating autophagy, and the AMPK/mTOR pathway is one of the signaling pathways associated with the antiapoptotic effects of Rg1 [55]. Rg1 has no obvious effect on megakaryocytes in the spleen of CY-induced mice [27] but can inhibit platelet activation by inhibiting the ERK pathway and attenuate arterial thrombosis [56]. In addition, Rg1 can reduce the infiltration of eosin-

ophils and mast cells in a mouse model of allergic rhinitis [57].

CLPs comprise another important branch of developing HPCs that have the potential to differentiate and develop into T cells, B cells, and natural killer (NK) cells [58]. Rg1 increases the proportion of T helper (Th) cells among total T cells and increases NK cell activity in the mouse spleen [5]. Specifically, Rg1 directly enhances the Th cell response without the participation of antigen-presenting cells (APCs) by increasing the IL-4 and IL-2 levels and reducing the IFN- γ levels to reduce the T helper type 1 (Th1) cell population and increase the T helper type 2 (Th2) cell population in the spleen [59]. In a murine sepsis model, Rg1 increases the neutrophil count in the abdominal cavity and inhibits lymphocyte apoptosis in the thymus and spleen [60].

In total, various in vitro cell stimulation experiments and in vivo animal disease models have shown that Rg1 could regulate the development of myeloid and lymphoid progenitor cells and affect the activity and secretion of mature blood cells, and the progeny of HPCs has also been shown to regulate HPC behavior, which suggests that Rg1 may effectively regulate both innate and adaptive immunity (Figure 7).

6. Rg1 May Indirectly Regulate the HSC Niche

Niches with various functions exist in different areas of the bone marrow. For example, the endosteal niche can support the quiescence and maintenance of HSCs, whereas the arteriolar niche maintains quiescent HSCs, and the sinusoidal niche supports the cycling of HSCs [61, 62]. New studies have also indicated that HSCs in perisinusoidal niches are protected from aging [63]. Moreover, MSCs, the vasculature, and nerve fibers can maintain quiescent HSCs and/or control HPC differentiation through cell-to-cell communication within the niche [64]. Importantly, Rg1 may be able to

finding suggests that Rg1 may also antagonize bone marrow adipogenesis and thereby benefits the hematopoietic microenvironment and protects HSCs.

In summary, in the bone marrow niche, Rg1 may alleviate HSC senescence through MSCs, regulate endothelial cells to expand HSCs, activate Schwann cells to maintain quiescent HSCs, and protect HSCs by inhibiting the formation of adipocytes (Figure 8). However, the relevant direct evidence must be verified by experimental data.

7. Conclusions and Remarks

Through the use of bioinformatics and molecular docking methods to analyze the molecular pharmacological mechanism through which Rg1 regulates HSCs/HPCs, we predicted that GVHD is a possible disease target of Rg1 therapy and that ACE is a potential target protein through which Rg1 regulates the proliferation and differentiation of HSCs/HPCs. A review of the literature also showed that Rg1 may regulate HSC proliferation and can activate extramedullary HSCs to migrate to the bone marrow. These results suggest a new strategy for HSC expansion in vitro and a new method for improving the HSC homing rate and alleviating GHVD in HSC transplantation in vivo. Moreover, the ability of Rg1 to alleviate HSC aging and regulate HPC development suggests that Rg1 exerts direct effects on the maintenance of HSCs/HPCs. However, whether Rg1 can promote the proliferation of HSCs without affecting their differentiation in vitro and whether Rg1 can enhance the HSC homing rate while reducing GVHD during HSC transplantation in vivo are worth further comprehensive exploration.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Fang He and Guanping Yao share first authorship.

Acknowledgments

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Supplementary Materials

Supplementary Material 1: putative targets of Rg1 identified using TargetNet. Supplementary Material 2: putative targets of Rg1 identified using SwissTargetPrediction. Supplementary Material 3: genes involved in HSC proliferation. Supplementary Material 4: genes involved in HSC migration. Supplementary Material 5: genes involved in HSC differentiation. Supplementary Material 6: genes involved in HPC differentiation. Supplementary Material 7: databases used in this study. (*Supplementary Materials*)

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