Review

Epigenetic regulations of hematopoietic stem cells ageing and the regulation of traditional Chinese medicine

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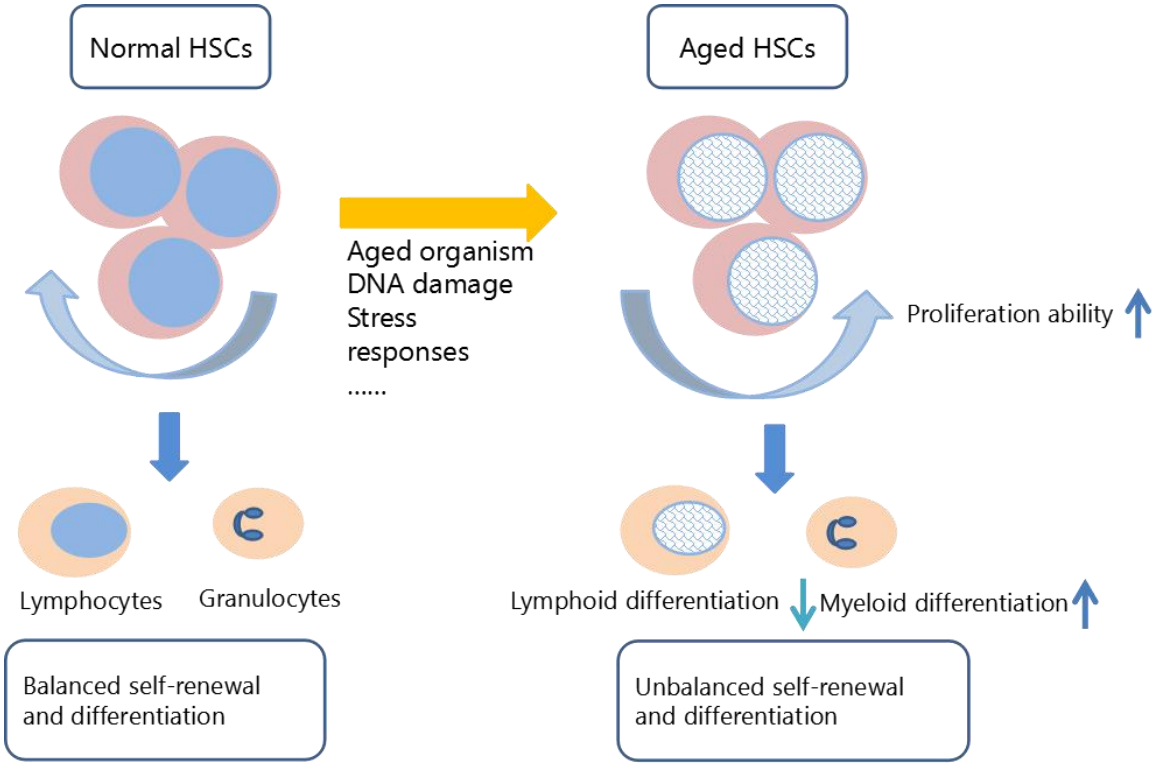
Highlights:

The current progress in the study of DNA methylation and histone modifications regulating HSCs ageing and the epigenetic role of TCM in the treatment of hematological malignancies were summarized.



Epigenetic regulations of hematopoietic stem cells ageing and the regulation of traditional Chinese medicine

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Abstract: Age-related defects in stem cells can limit proper tissue maintenance and hence contribute to a shortened lifespan. Given the essential role of a stem cell to self-renew, differentiate, and proliferate, it is no wonder that they are critically important to an organism during development and to tissue self-healing and regeneration. Hematopoietic stem cells (HSCs), as one of adult stem cells, have been well characterized recently. However, the regulation mechanism of HSCs ageing remains unclear. Epigenetic alterations are now recognized as a hallmark of mammalian ageing and involved in HSCs ageing. In addition, recent studies showed that HSCs ageing is related to hematological malignancies. Therefore, epigenetic modifiers maybe novel targets for hematological malignancies therapy. Traditional Chinese medicine (TCM) has been used to treat hematological malignancies for centuries. Here, we aim to review the current progress in the study of epigenetic changes regulating HSCs ageing, particularly focusing on the epigenome and its regulators in ageing HSCs. Furthermore, we will talk about the potential of TCM in treating hematological malignancies by regulating epigenetic changes.

Key words: Hematopoietic stem cells, Ageing, Epigenetics, Traditional Chinese medicine.

摘要

因为衰老而导致的干细胞功能缺陷会影响组织的稳态，并最终导致机体寿命缩短。干细胞具有自我更新和增殖分化能力，在机体生长发育过程中对组织的自我修复及再生无疑是至关重要的。成体干细胞中研究比较透彻的干细胞是造血干细胞(HSCs)，但是对其自身衰老调控的机制尚不清楚。表观修饰的改变被认为是哺乳动物衰老的标志之一，并且参与了 HSCs 的衰老过程。另外，近期的研究发现 HSCs 的衰老与血液肿瘤密切相关，因此表观遗传调控者或许可以成为治疗血液肿瘤的新靶点。长久以来中药一直被用于治疗血液肿瘤并且疗效确切，比如三氧化二砷。在这篇综述中，我们总结了表观修饰改变与 HSCs 衰老的关系，并且重点论述了 DNA 甲基化及组蛋白修饰调控 HSCs 衰老的机制。我们还总结了目前用于治疗血液肿瘤的部分中药，并对其表观遗传调控的角色进行了论述，旨在为开发新的药物提供依据。

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Introduction

Adult stem cells play essential roles in tissue self-healing and regeneration. Accumulating evidence shows that ageing is associated with the decrease and dysfunction of adult stem cells, which is thus considered to be one of important hallmarks of mammalian ageing [1]. Hematopoietic stem cells (HSCs), as one of adult stem cells, have been well functionally characterized recently. The capacity of long-term self-renewal and differentiation makes HSCs able to generate all mature lymphoid and myeloid lineages [2], including erythrocytes, granulocytes, platelets, macrophages and B- and T-lymphocytes. In young adults, it was shown that around 1000 active HSCs are responsible for the generation of all blood cell types [3], indicating a powerful self-renewal and differentiation potential. However, functions of HSCs are impaired with age (Figure 1), especially its ability to maintain the balance between self-renewal and differentiation, which is of vital importance to blood system [4, 5]. Compaired with its younger counterparts, aged HSCs show several characteristic phenotypes, including increased number, homing defect, impaired engraftment potential and repopulating capacity, and increased output of myeloid-biased progeny [6-10].

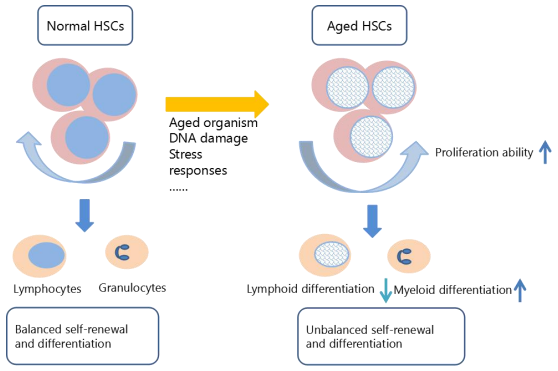

Figure 1 The characteristics of aged hematopoietic stem cells (HSCs).

Epigenetic regulations, including DNA methylation, histone modifications and non-coding RNAs interference, encompasses all heritable changes in gene expression that are not due to changes in DNA sequences [11, 12]. Such mechanism controls gene expression mainly via regulating chromatin structure and status, playing an important role in determining cells fate and ontogeny. Various epigenetic alterations has been recognized as another hallmark of mammalian ageing [1]. In the field of HSCs ageing, evidence shows that HSCs are not protected from ageing. Instead, loss of epigenetic regulation at the chromatin level may drive both functional attenuation of cells, as well as other manifestations of ageing, including the increased propensity for neoplastic transformation [5].

Traditional Chinese medicine (TCM) is a system of theories and therapies that was first documented in ancient Chinese classics dating back 2100 years. Epigenetics, combining genetics and environment, contributes to not only the stability of organisms but also their adaptability to the environment, which is consistent with the theory of human-environmental inter relation of TCM. Recently, epigenetics has been introduced to the area of TCM resulting in the hypothesis of an epigenetic role in the pharmacology of TCM prescriptions. Various traditional Chinese medicine has been shown to be safer and more effective for preventing cancer by targeting epigenetic landscape [13].

In this review, we aim to conclude that the altered functional potential of HSCs controlled at the epigenome level with age, focusing largely on DNA methylation, histone modifications, the two most characterized epigenetic marks. In addition, we will also talk about the epigenetic role of TCM in the treatment of hematological malignancies.

Epigenetic regulations and HSCs ageing

DNA methylation and HSCs ageing

DNA methylation is a potent epigenetic mark that promotes gene silencing. Methylation occurs at the 5-carbon position of cytosines (resulting in 5-methylcytosine or 5-mC) found in cytosine-phosphate-guanine dinucleotides (CpGs) through the action of the DNA methyltransferase (DNMT) family of proteins, including the de novo methyltransferases Dnmt3A and Dnmt3B and the maintenance methyltransferase Dnmt1. Dnmt1-deficient HSCs show self-renewal defects and decreased ability of multilineage hematopoiesis, indicating functions in both HSC self-renewal and differentiation [14, 15], while loss of Dnmt3A in HSCs impairs their differentiation and increases the number of bone marrow HSCs over serial transplantation [16]. Interestingly, loss of Dnmt3B has minimal effects on adult HSCs function. However, a more severe block in differentiation occurred in HSCs when Dnmt3B was ablated combined with Dnmt3A. Furthermore, it was also reported that Dnmt3B has distinct functions, given that Dnmt3B accounts for some HSCs differentiation in the absence of Dnmt3A [17, 18].

Apart from DNMT family, recently TET2 (the Ten-Eleven-Translocation 2), a member of TET family that encode enzymes modifying DNA by hydroxylating 5-methylcytosine (5mC) [19], has been shown to be essential to HSCs self-renewal and differentiation. Consistent with this, somatic loss-of-function mutations in TET2 are frequently found in patients with myeloid malignancies, for example myelodysplastic syndromes. TET2 loss leads to increased size of HSCs pool and TET2-deficient HSCs were shown to develop enhanced stem cell self-renewal in vivo competitive transplant assays [20, 21]. It was found that hypermethylation of Polycomb Repressive Complex 2 (PRC2) targets appears to

accompany forced proliferation and ageing, suggesting that DNA methylation plays a critical role in regulating the physiological ageing of HSCs [22, 23].

Histone modifications and HSCs ageing

Histone modification is one of the major covalent modifications that occurs at histone tails, including methylation, acetylation, sumoylation, phosphorylation, and ubiquitination, which have a critical role in dynamic modulation of chromatin structure and function, contributing to the regulation of cellular gene expression. These modifications involve in the chromatin remodeling and impact DNA accessibility. By taking advantage of highly purified HSCs, genome-wide comparisons of histone modifications between young and aged mouse HSCs were performed, enabling us to have a comprehensive understanding of the link between histone modification and HSC ageing. Recently, three key regulatory chromatin marks, H3K4me3 (trimethylation of Lys 27 of histone H3), H3K27me3 and H3K36me3, were chosen to assess epigenetic alterations in young and old HSCs [24]. Results from ChIP-seq suggest that aged HSCs exhibited broader H3K4me3 (an active mark) peaks, particularly in HSCs identity and self-renewal genes, showing a positive correlation with gene expression alteration. Furthermore, there was a strong positive correlation between changes of H3K4me3 and gene expression with age. Additionally, changes in H3K27me3 (a repressive mark) levels have also been described and similar to H3K4me3, H3K27me3 density around promoters expanded as well. Interestingly, $p16^{INK4a}$（a tumor suppressor protein encoded by *Cdkn2a*）is repressed by H3K27me3 both in young and old HSCs, while the increase of *Cdkn2a* is thought to be a hall mark of ageing for virtually all tissues [1].

Polycomb group (PcG) proteins are key epigenetic regulators of HSCs fate by maintain and propagate regulatory histone modifications [25]. Two Polycomb repressive complexes (PRCs), PRC1 and PRC2, have been shown to have distinct functions in the control of HSCs self-renewal, with PRC1 crucial to maintain gene repression while PRC2 crucial to initiate gene repression. PRC1 is the main H2A ubiquitin (H2Aub) ligase [26]. Within PRC1, Cbx family members functions in the modulation of the balance between HSCs self-renewal and differentiation [27]. Via H3K27me3 binding, overexpression of Cbx7 enhances HSCs self-renewal， while overexpression of Cbx2, Cbx4 or Cbx8 contributes to differentiation. In addition, overexpression of another member of PRC1, BMI1, in cord blood leads to long-term maintenance of human hematopoietic stem/progenitor cells [28].

The PRC2 complex is responsible for H3K27me3 through its enzymatic subunits EZH1 and EZH2 [29, 30]. PRC2 complex contains either EZH1 or EZH2, which is chromatin-modifying histone lysine methyltransferases [31]. Enforced expression of EZH2 in HSCs prevents their exhaustion during serial transplantations [32] and its conditional loss results in defect of muscle regenerative potential [33]. Much later it was shown that EZH2 in fact is frequently mutated in patients with myeloproliferative diseases. EZH2 deposits the epigenetic trimethyl mark that is recognized by the Cbx proteins contained in the PRC1 complex [27]. Whereas EZH1 and EZH2 have different chromatin binding properties, EZH1 can also provide enzymatic activity for the PRC2 complex. It was demonstrated that EZH1 is an important histone methyltransferase for HSCs maintenance [34]. EZH1 maintains repopulating HSCs in a slow-cycling, undifferentiated state, protecting them from senescence. Furthermore, Epigenetic and gene expression changes resulted from loss of EZH1 in aged HSCs showed that EZH1-mediated PRC2 activity catalyzes monomethylation and dimethylation of H3K27.

Members of the sirtuin family, NAD+-dependent protein deacetylases, particularly Sir2, have been investigated as potential anti-ageing factors. With seven homologs in mammals, the Sir2 family of histone deacetylases (HDACs) targets H4K16 and other proteins involved in regulating glucose and fatty acid metabolisms [35, 36]. Pharmacological inhibition of Cdc42 activity functionally rejuvenates aged HSCs, increases the percentage of polarized cells in an aged HSCs population, and restores the level and spatial distribution of histone H4 lysine 16 acetylation to a status similar to that seen in young HSCs [37], which leads to the hypothesis that epigenetic regulation by Sir2 family HDACs governed by the Cdc42 activity plays a role in HSC ageing [38]. Sirt3, a mammalian sirtuin, is highly enriched in HSCs [39]. Additionally, Sirt3 is dispensable for HSC maintenance at a young age but is essential at an old age. Importantly, Sirt3 upregulation in aged HSCs improves their regenerative capacity.

HSCs ageing and traditional Chinese medicine

HSCs typically show increased incidence of myeloid malignancies with age [40]. As pointed above, epigenetic alterations are considered as a hallmark of ageing and mutations in epigenetic regulator genes occur frequently in most hematological malignancies [41], with 20 – 22 % of de novo AML (acute myeloid leukemia) patients were found to have mutations in DNMT3A [42]. Simultaneously, epigenetic modifications are potentially reversible in contrast to genetic defects. In this context, remodeling of HSCs epigenome sheds a light on diseases preventive and therapeutic strategies. Indeed, 5-Azacytidine, a DMNT inhibitor, which epigenetically modulates various tumor suppressor genes, has been used for the myelodysplastic syndromes (MDS) and AML [43]. Chemopreventive nutritional polyphenols, such as soy, genistein, resveratrol, catechin, curcumin, are currently

evaluated for their ability to reverse adverse epigenetic marks in cancer (stem) cells to attenuate tumorigenesis-progression, prevent metastasis or sensitize for drug sensitivity [44].

Via a bioinformatic study, it was reported that 29.8% of 3294 TCM medicinals are epigenome- and miRNA-modulating by interacting with Polycomb group and methyl CpG-binding proteins [45]. Strikingly, within 200 TCM formulas, 99% of them are epigenome- and miRNA-interacting. Some herbal medicines are reported to target epigenetic modifiers and hence contribute to inhibit the proliferation of cancer cells (Table 1). *Feijoa sellowiana* extract, particularly flavone, exerts anti-cancer activities on hematological cancer cells [46]. Accompanied by p16 overexpression in human myeloid leukemia cells, *Feijoa* apoptotic activity correlates with the induction of HDAC inhibition.

Trichosanthin [47], tanshinone IIA [48], arsenic trioxide (ATO) [49, 50], yugan granule [51] and genistein [52] have been reported to have anti-cancer effects by targeting DNMTs in various cancer cells. Within these medicines, western blot and immunohistochemical analysis confirmed that tumor suppressors including p16 were markedly enhanced after treatment with a low concentration of ATO in human liver cancer cells. Additionally, ATO decreased the mRNA expression of DNMT 1 and also dose-dependently inhibited DNMT activity. Collectively, a low concentration of ATO induces demethylation of tumor suppressor genes by inhibition of DNMT and reactivates the partially/fully silenced genes in liver cancer cells [49]. ATO is also considered to be an efficient drug for the treatment of acute promyelocytic leukemia (APL). Researcher showed that the extent of total genomic DNA methylation of HL-60 cell decreased after ATO treatment, which is accompanied by reduced expression of DNMT3B with DNMT1 no significant change [50].

Curcumin, which is found in turmeric, functions as a strong anticancer agent in human prostate cancer cells through the modulation of HDACs [53-55]. Although the total HDAC activity was decreased upon CUR treatment, such treatment showed different effects on the protein expression of HDACs, increasing the expression of HDAC1, 4, 5, and 8 but decreasing HDAC3. Further analysis showed that CUR decreased the enrichment of H3K27me3 at the Neurog1 (a cancer methylation marker) promoter region as well as at the global level. Triptolide, which is the principal active ingredient of Chinese herb *Tripterygium wilfordii* Hook.F, has various functions such as antitumor properties. In the field of hematology, triptolide was shown to be able to inhibit the proliferation of multiple myeloma cells in a time- and dose-dependent manner, with induced G0/G1 cell cycle arrest and apoptosis [56]. In addition, the possible anti-myeloma mechanism of triptolide was to decrease histone H3K9 and H3K27 methylation via the downregulation of histone methyltransferase SUV39H1 and EZH2, respectively. Interestingly, by modulating histone H3-Lysine 9 (H3-K9) methylation and deacetylation, genistein is able to activate expression of several aberrantly silenced tumor suppressor genes as well, indicating a broad effect of Chinese herb on epigenetic landscape [58].

Taken together, these results suggest another mechanism to develop effective therapeutics based on epigenetics, and offer a strong support for the proposition that we can treat hematological malignancies resulting from HSCs ageing by taking advantage of the epigenetic role of TCM pharmacology.

Table 1 The epigenetic regulations of TCM on tumors

Targets	TCM or TCM active ingredients	Effects	References
DNMTs	Trichosanthin	↓ DNMTs activity in human breast cancer MDA-MB-231 cells	[47]
	Tanshinone IIA	↓ DNMT1 in HepG2 human hepatoma cells	[48]
	Arsenic trioxide	↑ DNMT1 and ↓ p16 in human hepatoma cells	[49]
		↓ DNMT3B in leukemiaHL-60 cells	[50]
	Yugan granule	↓ DNMT1, ↓ DNMT3A and ↓ DNMT3B in mice hepatoma cells	[51]
	Genistein	↓ DNMTs activity and ↑ p16 in KYSE 510 cells	[52]
	Curcumin	↑ Histone H3 acetylation in Raji, HL-60 and K562 cells	[54]
		↓ HDAC1 activity in HepG2 human hepatoma cells	[55]
	Triptolide	↓ SUV39H1 and EZH2 in multiple myeloma RPMI8226 cells	[56]
Histone modifications	Epigallocatechin-3-gallate (EGCG)	↓ HAT (histone acetyltransferase) activity in androgen-dependent prostate cancer cells	[57]
	Genistein	↑ Tumor suppressor genes (PTEN, CYLD, p53 and FOXO3a), remodeling the heterochromatic domains of their promoters in prostate cancer cells	[58]

Conclusion and perspectives

The role of epigenetic regulation in HSCs ageing is gradually becoming clearer. However, much work remains to be done to decipher the complete picture of epigenetic machineries that regulate HSCs ageing. In addition, HSC ageing is related to hematopoietic system malignancies. Therefore, targeting epigenetic genes may be a promising strategy to treat hematopoietic system malignancies. TCM is attractive to explore drugs targeting epigenetic modifiers. Whereas an increasing amount of TCM have been indentified to be effective in the treatment of various cancers in a epigenetic manner, more studies need to be carried out to assess the role of TCM in treating hematopoietic malignancies and other HSC-ageing-related diseases.

Competing interests

The authors declare that they have no competing interests.

References

1. Lopez-Otin C, Blasco MA, Partridge L, et al. The hallmarks of aging. Cell 2013, 153(6): 1194-1217.
2. Bryder D, Rossi DJ, Weissman IL. Hematopoietic stem cells: the paradigmatic tissue-specific stem cell. Am J Pathol 2006, 169(2): 338-346.
3. Catlin SN, Busque L, Gale RE, et al. The replication rate of human hematopoietic stem cells in vivo. Blood 2011, 117(17): 4460-4466.
4. Morrison SJ, Wandycz AM, Akashi K, et al. The aging of hematopoietic stem cells. Nature Medicine 1996, 2(9): 1011-1016.
5. Chambers SM, Shaw CA, Gatza C, et al. Aging hematopoietic stem cells decline in function and exhibit epigenetic dysregulation PLoS Biol 2007, 5(8): e201.
6. Florian MC, Dorr K, Niebel A, et al. Cdc42 activity regulates hematopoietic stem cell aging and rejuvenation. Cell Stem Cell 2012, 10(5): 520-530.
7. Dykstra B, Olthof S, Schreuder J, et al. Clonal analysis reveals multiple functional defects of aged murine hematopoietic stem cells. J Exp Med 2011, 208(13): 2691-2703.
8. Xing Z, Ryan MA, Daria D, et al. Increased hematopoietic stem cell mobilization in aged mice. Blood 2006, 108(7): 2190-2197.
9. Geiger H, de Haan G, Florian MC. The ageing haematopoietic stem cell compartment. Nat Rev Immunol. 2013, 13(5): 376-389.
10. Cho RH, Sieburg HB, Muller-Sieburg CE. A new mechanism for the aging of hematopoietic stem cells: aging changes the clonal composition of the stem cell compartment but not individual stem cells. Blood 2008, 111(12): 5553-5561.
11. Bannister AJ, Kouzarides T. Reversing histone methylation. Nature 2005, 436(7054): 1103-1106.
12. Hake SB, Allis CD. Histone H3 variants and their potential role in indexing mammalian genomes: The "H3 barcode hypothesis". Proc Natl Acad Sci 2006, 103(17): 6428-6435.
13. Hun Lee J, Shu L, Fuentes F, et al. Cancer chemoprevention by traditional chinese herbal medicine and dietary phytochemicals: targeting Nrf2-mediated oxidative stress/anti-inflammatory responses, epigenetics, and cancer stem cells. J Tradit Complement Med 2013, 3(1): 69-79.
14. Broske AM, Vockentanz L, Kharazi S, et al. DNA methylation protects hematopoietic stem cell multipotency from myeloerythroid restriction. Nat Genet 2009, 41(11): 1207-1215.
15. Trowbridge JJ, Snow JW, Kim J, et al. DNA Methyltransferase 1 is Essential for and Uniquely Regulates Hematopoietic Stem and Progenitor Cells. Cell Stem Cell 2009, 5(4): 442-449.
16. Challen GA, Sun D, Jeong M, et al. Dnmt3a is essential for hematopoietic stem cell differentiation. Nat Genet 2011, 44(1): 23-31.
17. Mayle A, Sun D, Jeong M, et al. Dnmt3b Has Few Specific Functions In Adult Hematopoietic Stem Cells But Shows Abnormal Activity In The Absence Of Dnmt3a. Blood 2013,122(4): 734.
18. Challen G, Sun D, Mayle A, et al. Dnmt3a and Dnmt3b Have Overlapping and Distinct Functions in Hematopoietic Stem Cells. Cell Stem Cell 2014, 15(3): 350-364.
19. Myunggon K, Yun H, Jankowska AM, et al. Impaired hydroxylation of 5-methylcytosine in myeloid cancers with mutant TET2. Nature 2010, 468(7325): 839-843.
20. Moran-Crusio K, Reavie L, Shih A, et al. Tet2 loss leads to increased hematopoietic stem cell self-renewal and myeloid transformation. Cancer Cell 2011, 20(1): 11-24.
21. Myunggon K, Bandukwala HS, Jungeun A, et al. Ten-Eleven-Translocation 2 (TET2) negatively regulates homeostasis and differentiation of hematopoietic stem cells in mice. Proc Natl Acad Sci 2011, 108(35): 14566-14571.
22. Koide S, Wendt GR, Iwama A. Epigenetic regulation of hematopoietic stem cells. Inflamm Regen 2013, 33(4): 197-202.
23. Beerman I, Bock C, Garrison BS, et al. Proliferation-dependent alterations of the DNA methylation landscape underlie hematopoietic stem cell aging. Cell Stem Cell 2013, 12(4): 413-425.
24. Sun D, Luo M, Jeong M, et al. Epigenomic profiling of young and aged HSCs reveals concerted changes during aging that reinforce self-renewal. Cell Stem Cell 2014, 14(5): 673-688.
25. Schuettengruber B, Chourrout D, Vervoort M, et al. Genome Regulation by Polycomb and Trithorax Proteins. Cell 2007, 128(4): 735-745.
26. Wang H, Wang L, Erdjument-Bromage H, et al.

Role of histone H2A ubiquitination in Polycomb silencing. Nature 2004, 431(7010): 873-878.
27. Klauke K, Radulović V, Broekhuis M, et al. Polycomb Cbx family members mediate the balance between haematopoietic stem cell self-renewal and differentiation. Nat Cell Biol 2013, 15(4): 353-362.
28. Rizo A, Dontje B, Vellenga E, et al. Long-term maintenance of human hematopoietic stem/progenitor cells by expression of BMI1. Blood 2008, 111(5): 2621-2630.
29. Schuettengruber B, Cavalli G. Recruitment of polycomb group complexes and their role in the dynamic regulation of cell fate choice. Development 2009, 136(21): 3531-3542.
30. Simon JA, Kingston RE. Mechanisms of polycomb gene silencing: knowns and unknowns. Nat Rev Mol Cell Biol 2009, 10(10): 697-708.
31. Margueron R, Reinberg D. The Polycomb complex PRC2 and its mark in life. Nature 2011, 469(7330): 343-349.
32. Kamminga LM, Bystrykh LV, Aletta DB, et al. The Polycomb group gene Ezh2 prevents hematopoietic stem cell exhaustion. Blood 2006, 107(5): 2170-2179.
33. Samuel W, Dhamayanthi P, Patrick B, et al. Ezh2 maintains a key phase of muscle satellite cell expansion but does not regulate terminal differentiation. J Cell Sci 2013, 126(2): 565-579.
34. Isabel H, Antonio HM, Jose Manuel L, et al. Ezh1 Is Required for Hematopoietic Stem Cell Maintenance and Prevents Senescence-like Cell Cycle Arrest. Cell Stem Cell 2012, 11(5): 649-662.
35. Houtkooper RH, Pirinen E, Auwerx J. Sirtuins as regulators of metabolism and healthspan. Nat Rev Mol Cell Biol 2012, 13(4): 225-238.
36. Hall JA, Dominy JE, Lee Y, et al. The sirtuin family's role in aging and age-associated pathologies. J Clin Invest 2013, 123(3): 973-979.
37. Florian MC1, Dörr K, Niebel A et al. Cdc42 activity regulates hematopoietic stem cell aging and rejuvenation. Cell Stem Cell 2012, 10(5): 520-530.
38. Oshima M, Iwama A. Epigenetics of hematopoietic stem cell aging and disease. Int J Hematol 2014, 100(4): 326-334.
39. Brown K, Xie S, Qiu X, et al. Sirt3 reverses aging-associated degeneration. Cell Rep 2013, 3(2): 319-327.
40. Pang WW, Price EA, Debashis S, et al. Human bone marrow hematopoietic stem cells are increased in frequency and myeloid-biased with age. Proc Natl Acad Sci USA 2011, 108(50): 20012-20017.
41. Chung YR, Schatoff E, Abdel-Wahab O. Epigenetic alterations in hematopoietic malignancies. Int J Hematol 2012, 96(4): 413-427.
42. Yan XJ, Xu J, Gu ZH. et al. Exome sequencing identifies somatic mutations of DNA methyltransferase gene DNMT3A in acute monocytic leukemia. Nat Genet 2011, 43(4): 309-315.
43. Müller AM, Florek M. 5-Azacytine/5-Azaci -tidine. Recent Results Cancer Res 2014, 201(2): 299-324.
44. Vanden Berghe W. Epigenetic impact of dietary polyphenols in cancer chemoprevention: Lifelong remodeling of our epigenomes. Pharmacol Res 2012, 65(6): 565-576.
45. Hsieh HY, Chiu PH, Wang SC. Epigenetics in traditional chinese pharmacy: a bioinformatic study at pharmacopoeia scale. Evid Based Complementary Alternat Med 2011; 2011:816714.
46. Bontempo P, Mita L, Miceli M, Doto A, et al. Feijoa sellowiana derived natural flavone exerts anti-cancer action displaying hdac inhibitory activities. Int J Biochem Cell Biol, 2007, 39(10): 1902-1914.
47. Hua F, Shan BE, Zhao LM, et al. Trichosanthin inhibited the proliferation of MDA-MB-231 cell and reversed the methylation of sykgene . Cancer 2009, 29(10): 944-949.
48. Tian XF, Tao YM, Fang Y, et al. Study of salviae Miltiorrhize extract on DNA demethylation in HepG2 cells. J Hunan Univ Tradit Chin Med 2009, 29(1): 13-15.
49. Cui X, Wakai T, Shirai Y, et al. Arsenic trioxide inhibits dna methyltransferase and restores methylation-silenced genes in human liver cancer cells. Hum Pathol 2006, 37(3): 298 – 311.
50. Peng CY. The epigenetic mechanisms of arsenic trioxide anti leukemia. Shantou University 2009.
51. Lv F, Shao ZY, Xie ZL, et al. Suppressive effect and the mechanism of epigenetics of Yugan granule on liver cancer in rats. Asia-Pac Tradit Med 2008, 4(10): 26-28.
52. Fang MZ, Chen D, Sun Yet al. Reversal of hypermethylation and reactivation of p16INK4a, RAR β , and MGMT genes by genistein and other isoflavones from soy. Clin Cancer Res 2005, 11(19): 7033-7041.
53. Shu L, Khor TO, Lee JH, et al. Epigenetic cpg demethylation of the promoter and reactivation of the expression of neurog1 by curcumin in prostate lncap cells. AAPS J 2011, 13(4): 606-614.
54. Wang Y, Hu JB, Chen Y, et al. Curcum in causes histone acetylation enhancement in Raji, HL- 60 and K562 cell lines. Chin Pharm Bull 2006, 22(2): 164-167.
55. Lv BH, Zhang L, Zhu CC, et al. Inhibition of curcumin on histone deacetylase and expression promotion of P21WAF1 /CIP1 in HepG2 cells. China J Chin Mater Med 2007, 32(19): 2051-2055.
56. Zhao F, Chen Y, Li R, et al. Triptolide alters histone H3K9 and H3K27 methylation state and

induces G0/G1 arrest and caspase-dependent apoptosis in multiple myeloma in vitro. Toxicology 2010, 267(1): 70-79.
57. Lee YH, Kwak J, Choi HK, et al. EGCG suppresses prostate cancer cell growth modulating acetylation of androgen receptor by anti-histone acetyltransferase activity. Int J Mol Med 2012, 30(1): 69-74.
58. Kikuno N1, Shiina H, Urakami S, et al. Genistein mediated histone acetylation and demethylation activates tumor suppressor genes in prostate cancer cells. Int J Cancer 2008, 123(3): 552-560.

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