Title:	Nanotherapeutic Regulation of Polycomb Protein Mediated Epigenetic Retardation of Acute Myeloid Leukemia
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Abstract: Acute Myeloid Leukemia (AML) is heterogeneous hematological malignancy which initiate in bone marrow microenvironment (BMM) affecting myeloid lineage of hematopoietic stem cells. AML pathophysiology comprise of genetic factors like fusion oncogenes, MLL-AF9 and AML1-ETO which contribute to 20-30% AML occurrence. AML reports abnormal epigenetic patterns of histone methylation and ubiquitination wherein polycomb proteins EzH1/2 tri-methylate histone H3 at lysine 27 position, which serves as epigenetic hallmark to recruit other polycomb proteins, Ring1A/B and Bmi1 to monoubiquitinate histone H2A at lysine 119 position. This leads to histone compaction, and deregulation of tumor suppressor INK4A/ARF locus resulting in uncontrolled cell proliferation. The overexpression of Bmi1, Ring1B, EzH1 and EzH2 in AML and their involvement in disease onset, maintenance, and relapse reckon them as appealing targets for anti-AML therapies. In addition, transcription factor, C-Myb regulate polycomb proteins through its promoter activity and this indispensable relationship regulate several genes in AML. Moreover, the limitations of available AML treatments mandate the development of new and innovative therapeutic modules wherein the obstruction of polycomb proteins-based epigenetic regulations through nanoformulations pledge for safe, efficient, innovative, and superior alternative AML therapies. The first chapter of the thesis focuses on the review of literatures contributed towards the polycomb signalling and their regulation in AML pathogenesis and therapeutic interventions with the development of novel anti-AML therapeutics based on epigenetic regulation of AML to circumvent the limitations of available therapies through nanotechnology approach. The second chapter is dedicated for the methodologies to synthesize, modify, and characterize the nanoparticles along with study design to execute the evaluation of in vitro and in vivo anti-AML efficacies in cell line-induced xenografts and patient-derived xenografts (PDX). The third chapter of thesis manifests the exploration of Bmi1-specific inhibitor and siRNA-based nanoformulations as anti-AML therapeutics under in vitro and in vivo AML xenografts. The first part of third chapter explores Bmi1-specific inhibitor, PRT4165-encapsulated human serum albumin (HSA) nanoparticles (PRT@HSANPs) to exhibit an enhanced anti-AML therapy. PRT@HSANPs improve the solubility, stability, and release pattern of PRT4165 which in turn ameliorate the in vitro anti-AML effects with caspase 3 dependent apoptosis pathway and induce Bmi1 downregulation through ubiquitin-proteasome pathway (UPP). PRT@HSANPs suppress CD45 + leukemia stem cells (LSCs) population and stimulate CD11b+ myeloid differentiation markers in the bone marrow of cell line- induced xenograft mice. The second part of third chapter demonstrate RNA interference (RNAi)-based therapy using Bmi1 siRNA wherein polyethyleneimine (PEI)-stabilized Bmi1 siRNA-entrapped HSA nanocarriers (si-Bmi1@HSANCs) display superlative epigenetic regulation-based therapy. si- Bmi1@HSANCs protect Bmi1 siRNA to increase the transfection efficiency through caveolae- mediated endocytosis and exhibit enhanced cytotoxicity through caspase 3 dependent apoptosis along _____ 1_____ with Bmi1 downregulation through UPP. C-Myb directly regulate Bmi1 through promoter binding between -235 to +43 and -111 to +43. si-Bmi1@HSANCs demonstrate a decrease in CD45 + LSCs and increase in CD11b + population in bone marrow of AML xenografts. The fourth chapter is dedicated towards the active targeting of AML phenotypes with DNA aptamers. The first half of the chapter deals with CD123 (ZW25)-directed EzH2 siRNA-entrapped HSA nanoparticles (si-EzH2@HNPs@ZW25) which exhibit enhanced transfection efficiency and cytotoxicity to MLL-AF9 retroviral-induced in vitro cells along with caspase 3 dependent apoptosis. si- EzH2@HNPs@ZW25 suppress c-Kit + LSCs and promote CD11b + and Gr-1 + differentiating population under MLL-AF9-induced xenografts and CD34 + CD38 - PDX models. si-EzH2@HNPs@ZW25 suppress C-Myb mediated EzH2 regulation in AML which is reported to be indispensable for AML pathology. The next half of the chapter extends the active targeting of AML with CD33 (S30)-directed EzH1-encapsulated HSA nanoparticles (si-EzH1@HNP@S30) which display the increased transfection efficiency and cytotoxicity through caspase 3 dependent apoptosis towards AML1-ETO9a retroviral- induced in vitro cells. si-EzH1@HNP@S30 diminish c-Kit + LSCs and stimulate CD11b + and Gr-1 + population under AML1-ETO9a-induced xenografts and CD34 + CD38 - PDX models. C-Myb directly regulate EzH1 expression wherein si-EzH1@HNP@S30 abrogate this indispensable regulation in AML pathology. The fifth chapter operates around the bone marrow targeting through nanoparticles which is an unexplored field in AML therapy development wherein the bone homing bisphosphonates, Alendronic acid and Ibandronic acid provide such targeting when surface functionalized. The first segment of fifth chapter renders with the development of alendronic acid-functionalized PRT415-encapsulated HSA nanoparticles (PRT@HSANPs@ALD) which exhibit excellent bone matrix binding and BMM localization. PRT@HSANPs@ALD deliver Ring1B inhibitor (PRT4165) in AML1-ETO9a retroviral- induced in vitro cells and suppress c-Kit + LSCs and promote CD11b + and Gr-1 + population in the bone marrow of AML1-ETO9a-induced xenografts and CD34 + CD38 - PDX mice. PRT@HSANPs@ALD downregulate Ring1B, H2AK119ubi and C-Myb in vitro and in vivoAML1-ETO9a condition wherein C-Myb directly regulate Ring1B and PRT@HSANPs@ALD abolish this crucial relationship to control the AML pathology. In the last section of fifth chapter demonstrate the Ibandronic acid-functionalized and C-Myb siRNA-encapsulated Vitamin D nanoemulsion (si-Myb@NVD@IBD) efficient binding to bone matrix and its localization to BMM. si-Myb@NVD@IBD exhibit superior anti-AML therapeutics in MLL-AF9 retroviral-induced in vitro cells along with restricting the proliferation of c-Kit + LSCs and promoting the CD11b + and Gr-1 + population in the bone marrow of AML1-ETO9a-induced xenografts and CD34 + CD38 - PDX mice. Further analysis enlightens C-Myb and Survivin downregulation by si-Myb@NVD@IBD wherein nanoformulations abolish C-Myb-mediated transcriptional regulation of Survivin to control the AML pathology.

_____ 2_____ In addition, adoptive transfer of NK cells suppresses AML and inhibition of EzH2 activate natural killer (NK) cells enhances the anti-AML immunotherapy. This approach is still under scrutiny due to limitations like production cost, vein-to-vein time, and complicated experimental procedures. Our nanoformulation, CD56 antibody-conjugated pSMP-EzH2 shRNA-encapsulated chitosan nanoparticles (pEzH2@CSNPs@CD56), selectively target and downregulate EzH2 in CD56 + NK cells in human peripheral blood which is better compared to retrovirally EzH2-downregulated in vitro NK (EzH2-) cells. The pEzH2@CSNPs@CD56 exhibit suppression of AML pathology as evident through the reduced splenomegaly and suppressed c-Kit + LSCs and upregulated CD11b + and Gr-1 + population in peripheral blood and bone marrow of AML1-ETO9a-induced xenograft mice. The activation CD56 + NK cells and increased CD38 + cells population by pEzH2@CSNPs@CD56 suggest granzyme B-induced caspase 3 dependent apoptosis pathway as superior NK cells-mediated anti-AML immunotherapy.

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