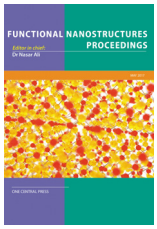
Available online at www.onecentralpress.com
One Central Press
proceedings webpage: www.onecentralpress.com/functional-nanostructures-proceedings



Therapeutic Targeting of the Leukaemic Fusion Gene RUNX1/ETO

H. ISSA[1], H. Blair[1], N Jyotsana[2], M. Heuser[2], O Heidenreich[1]

[1]NICR, Newcastle University, Brewery Lane, Newcastle upon Tyne, NE1 7RU, UK.
[2]Hannover Medical School, Carl-Neuberg-Str1, Hannover, 30625, Germany.

ABSTRACT

The t(8,21) translocation is the most prevalent chromosomal translocation in Acute Myeloid Leukaemia (AML), which leads to the expression of chimaeric RUNX1/ETO oncogene protein. Our previous work showed that RNAi-mediated RUNX1/ETO knockdown inhibits cell proliferation, reduces clonogenicity, causes G1 cell cycle arrest and impairs engraftment in immunocompromised host.

We have silenced RUNX1/ETO expression by an LNP/siRNA system in vitro and in vivo. Dlin-MC3-DMA lipid nanoparticles (LNP) have been previously shown to have high encapsulation efficiency of siRNA, low toxicity and desirable diameter and charge properties for oligonucleotide delivery.

To enhance the efficacy of RUNX1/ETO targeted siRNA, we introduced several modifications on the 2'-position of the ribose and on the phosphodiester backbone. In vitro studies demonstrated that the chemically modified siRNA substantially inhibited RUNX1/ETO and provided prolonged phenotypic and greater effect on AML cell lines in comparison with unmodified siRNA. Single treatment with modified LNP/siRNA induced a long-lasting inhibition of RUNX1/ETO in vitro and in t(8,21) AML primary patient cells. Pharmacodynamic studies showed that a single dose of LNP/siRNA by systematic delivery route provides global body distribution in immunocompromised mice including leukaemic tissues, liver, spleen, bone marrow and brain.

Knockdown efficacy of the LNP/siRNA system was also examined in a xenotransplantation model. Harvested human leukaemic cells from siRNA-treated Rag2-/- γc-/- mice showed substantial reduction of RUNX1/ETO level with low dose of LNP/siRNA. This knockdown was associated with significant downregulation of RUNX1/ETO target genes such as CCND2 and TERT. RUNX1/ETO depletion also severely impaired the clonogenic potential of the harvested leukaemic cells from LNP/siRNA treated mice and triggered senescence. Notably, only three doses of LNP/RUNX1/ETO siRNA entirely prevented the expansion of leukaemic cells in secondary transplanted recipients. LNP/siRNA mediated RUNX1/ETO depletion in vivo significantly enhanced the survival of immunocompromised mice. In line with these findings, RNA-seq indicated that in vivo depletion of RUNX1/ETO activates a myeloid differentiation programme.

Taken together, we have demonstrated that liposomal delivery of chemically modified RUNX1/ETO siRNA impairs RUNX1/ETO-driven transcriptional networks and therewith associated leukaemic self-renewal function and initiates differentiation, which may have a therapeutic potential.