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主　論　文　の　要　旨

論文題目　Effect of SHARP1 Knockdown and PLEKHA7 Re-expression on the Growth and Behavior of Acute Myeloid Leukemia Cells induced by Functional Lipid Nanoparticles
（機能性脂質ナノ粒子によって誘導された急性骨髄性白血病細胞の成長と挙動に対する SHARP1 ノックダウンおよび PLEKHA7 再発現の影響）

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論　文　内　容　の　要　旨

This study focuses on assessing the potential therapeutic activity of SHARP1 knockdown using multifunctionally targeted delivery system of therapeutic siRNA against MLL-AF6 AML cells and evaluating the effect of PLEKHA7 protein-based nanodelivery on the behavior alteration and growth retardation of AML cells.

It is gradually being understood that these types of cancer cells are massively resistant to eradication due to high genetic mutations beside also they ultimately cause patient relapse. Cytarabine and Anthracycline combination is still regarded as a standard treatment of AML while the clinical efficacy of this combination is poor and results in the death of millions of patients annually. Consequently, identifying new treatments for AML may contribute substantially towards improving the survival of patients with this type of cancer. Therefore, to demonstrate a new way for AML therapy, herein, we utilize cRGD-mediated PEGylated cationic lipid nanoparticles as a biodegradable nanoplatform to co-deliver siRNA/BTZ with targeted SHARP1 knockdown, demonstrating a potential therapeutic option for MLL-AF6 AML. On the other hands, we investigate the effect of PLEKHA7 protein on the behavior and growth of AML cells and demonstrate how the existence of PLEKHA7 could alter AML cell characterizations. Since the role of PLEKHA7 in AML has not been studied previously, we attempt to demonstrate the correlation between PLEKHA7 expression and the incidence of AML growth. We further synthesize bioengineered nanoparticles for PLEKHA7 delivery (Lipo-PLEKHA7-PEG-cRGD) to regulate the induction of

AML behavior alteration and growth retardation in order to recruit a potential target for AML modulation.

In summary, we investigated SHARP1 knockdown in ML-2 cells in stimulating apoptosis to determine the oncogenic role of SHARP1 as an MLL-AF6-dependent leukemogenic driver in MLL-AF6 AML growth and maintenance, as well as the application of multifunctionally bioengineered nanoparticles in SHARP1 downregulation; thus, indicating a potential therapeutic strategy for human MLL-AF6 AML therapy. We have fabricated a state-of-the-art biodegradable nanoplatform for siRNA/BTZ co-delivery with targeted SHARP1 knockdown, demonstrating a potential therapeutic option for MLL-AF6 AML. This efficient co-delivery of siRNA and BTZ resulted in remarkable inhibition in cell viability and clonogenic growth as well as triggering apoptosis *in vitro.* We hypothesized that SHARP1 downregulation induced the accumulation of non-functional MLL-AF6, DOT1L, MEN1, and LEDGF fusion proteins, preventing MLL-AF complex formation and downregulating RAS-GTP and Bcl-2 expression, consequently triggering apoptosis. The BTZ combination substantially augmented therapeutic synergy and enhanced apoptotic events. The results demonstrated the effects of SHARP1 downregulation on DOT1L and MLL-AF6 expression and highlighted a new vital oncogenic role of SHARP1 in MLL-AF6 AML growth and maintenance. Taken together, Lipo-siRNA-BTZ-PEG-cRGD are multifunctional particles that reveal versatile regulatory mechanisms, including SHARP1 silencing, MLL-AF6/DOT1L inhibition, p53 activation, RAS suppression, proteasome inhibition, and apoptosis induction. In addition, we have firstly studied the PLEKHA7 expression in leukemic cells to assess their growth capability affected by the restoration of PLEKHA7 in the cells. The efficacy of PLEKHA7-loaded cRGD-mediated PEGylated cationic lipid nanoparticles for efficient PLEKHA7 delivery in leukemic cells as well as the effect of PLEKHA7 on the regulated induction of AML behavior alteration and growth retardation were investigated. PLEKHA7 re-expression inhibited cell proliferation, diminished colony-forming ability and reinforced the incidence of growth retardation without apoptosis in AML cell lines. PLEKHA7 regulated the restoration of cell surface adhesion and integrity during normal homeostasis. To our knowledge, the role of PLEKHA7 in AML had not been studied previously and our data could be exploited for further mechanistic studies and insights into altering human AML behavior and attenuating its aggressiveness. Our findings uncover the important function of PLEKHA7 as a adhesion relative modulator and growth attenuator in AML via an in vitro study on new bioengineered smart nanoparticles that are well-characterized,

cRGD-conjugated with thiolated PEG (NHS-PEG6-maleimide). This study revealed that PLEKHA7 re-expression could restore apical ZA integrity and cellular adhesion and attenuate AML growth rate. Altogether, these findings are beneficial and essential for developing PLEKHA7 nanomedicines for AML regulation and reprogramming.

We summarize the dissertation in 5 chapters including;

Chapter 1 is a general introduction of the research background, such as overview on AML, the vital roles of SHARP1 and PLEKHA7 in AML, as well as a review of the importance, usefulness, and significance of nanotechnology-based therapeutics, drug delivery systems and passive and active targeting for therapeutic drug delivery.

Chapter 2 represents the materials and methods which are utilized in this study.

Chapter 3 describes a comprehensive *in vitro* analysis on new multifunctional bioengineered smart nanoparticles using well-characterized cRGD-conjugated thiolated PEG (NHS-PEG6-maleimide) to effectively co-deliver siRNA/BTZ for targeted SHARP1 silencing in MLL-AF6 AML cells. This chapter discusses the effects of SHARP1 downregulation on DOT1L and MLL-AF6 expression and highlights a new vital oncogenic role of SHARP1 in MLL-AF6 AML growth and maintenance.

Chapter 4 demonstrates the effect of PLEKHA7, an apical adherens junction protein, using cRGD-mediated PEGylated cationic liposomal nanoparticles as a nanocarrier to investigate the regulated induction of AML behavior alteration and growth retardation. PLEKHA7 re-expression could diminish colony-forming ability and reinforce the incidence of growth retardation without apoptosis in AML cell lines. Furthermore, PLEKHA7 could regulate the restoration of cell surface adhesion and integrity during normal homeostasis.

Chapter 5 is the conclusion of this dissertation.

Eventually, the study aims to demonstrate the potential therapeutic effect of SHARP1 knockdown on MLL-AF6 AML cells and also to evaluate the effect of PLEKHA7 protein on the behavior and growth of AML cells using the targeted nanodelivery system (Lipo-PEG-cRGD) of therapeutic siRNA and PLEKHA7 into AML cells.