香港浸會大學
HONG KONG BAPTIST UNIVERSITY

HKBU Scholars

DOCTORAL THESIS

Aptamer-functionalized lipid nanoparticles targeting osteoblasts as a novel RNA Interference-based bone anabolic strategy

Liang, Chao

Date of Award:
2016

Link to publication

General rights
Copyright and intellectual property rights for the publications made accessible in HKBU Scholars are retained by the authors and/or other copyright owners. In addition to the restrictions prescribed by the Copyright Ordinance of Hong Kong, all users and readers must also observe the following terms of use:

• Users may download and print one copy of any publication from HKBU Scholars for the purpose of private study or research
• Users cannot further distribute the material or use it for any profit-making activity or commercial gain
• To share publications in HKBU Scholars with others, users are welcome to freely distribute the permanent URL assigned to the publication

Download date: 08 Dec, 2021

Abstract

Osteoporosis remain major clinical challenges. RNA interference (RNAi) provides a promising approach for promoting osteoblastic bone formation to settle the challenges. However, the major bottleneck for translating RNAi with efficacy and safety to clinical bone anabolic strategy is lack of osteoblast-specific delivery systems for osteogenic siRNAs.

Previously, we developed a targeting system involving DOTAP-based cationic liposomes attached to oligopeptides $(AspSerSer)_6$, (also known as $(DSS)_6$), which had good affinity for bone formation surface. Using this system, osteogenic Pleckstrin Homology Domain Containing, Family O Member 1 (*Plekho1*) siRNA could be specifically delivered to bone formation surface at tissue level and promoted bone formation in osteopenic rodents. However, concerns still exist regarding indirect osteoblast-specific delivery, detrimental retention in hepatocytes, mononuclear phagocyte system (MPS)-induced dose reduction and inefficient nanoparticle extravasation.

Aptamers, selected by cell-based Systematic evolution of ligands by exponential enrichment (cell-SELEX), are single-stranded DNA (ssDNA) or RNA which binds to target cells specifically by distinct tertiary structures. By performing positive selection with osteoblasts and negative selection with hepatocytes and peripheral

blood mononuclear cells (PBMCs), we aimed to screen an aptamer that could achieve direct osteoblast-specific delivery and minimal hepatocyte and PBMCs accumulation of *Plekho1* siRNAs. In addition, lipid nanoparticles (LNPs) have been widely used as nanomaterials encapsulating siRNA due to their small particle size below 90 nm. Polyethylene glycol (PEG) as the mostly used hydrophilic polymer, could efficiently prevent LNPs from MPS uptake. So, LNPs with PEG shielding could serve as siRNA carriers to realize efficient extravasation from fenestrated capillaries to osteoblasts and help reduce MPS uptake of the siRNAs.

Recently, we screened an aptamer (CH6) by cell-SELEX specifically targeting both rat and human osteoblasts and developed the aptamer-functionalized LNPs encapsulating osteogenic *Plekho1* siRNA, *i.e.*, CH6-LNPs-siRNA. Our results demonstrated that CH6-LNPs-siRNA had an average particle size below 90 nm and no significant cytotoxicity *in vitro*. CH6 aptamer facilitated osteoblast-selective uptake of *Plekho1* siRNA and gene silencing *in vitro*.

In this study, we further found that CH6 aptamer facilitated the bone-specific distribution of siRNA by biophotonic imaging and quantitative analysis. Immunohistochemistry results showed that CH6 achieved *in vivo* osteoblast-specific delivery of *Plekho1* siRNA. Dose-response experiment indicated that CH6-LNPs-siRNA achieved almost 80% gene knockdown at the siRNA dose of 1.0 mg/kg and maintained 12 days for over 50% gene silencing. microCT, bone

histomorphometry and mechanical testing confirmed that CH6 facilitated bone formation, leading to improved bone micro-architecture, increased bone mass and enhanced mechanical properties in osteoporotic rodents. Furthermore, CH6-LNPs-siRNA achieved better bone anabolic action when compared to the previously developed $(AspSerSer)_6$-liposome-siRNA. There was no obvious toxicity in rats injected with CH6-LNPs-siRNA.

All these results indicated that osteoblast-specific aptamer-functionalized LNPs could act as a novel RNAi-based bone anabolic strategy and advance selectivity of targeted delivery for osteogenic siRNAs from tissue level toward cellular level.

In addition, the generation of ssDNA from double-stranded PCR products is an essential step in selection of aptamers in SELEX. We found that the size separation derived from unequal primers with chemical modification could be a satisfactory alternative to the classic magnetic separation.

Table of Contents

DECLARATION I

Abstract II

Acknowledgements V

Table of Contents VI

List of Tables XII

List of Figures XIII

List of Abbreviation XVI

Chapter 1 General introduction 1

1.1 Epidemiology of osteoporosis 1

1.2 Challenge for reversing osteoporosis 1

1.3 Bottleneck for RNAi-based bone anabolic strategy 2

1.4 Exploration of promising targets for RNAi-based bone anabolic strategy . 3

1.4.1 Candidate molecular targets in BMP signaling pathway 4

1.4.2 Candidate molecular targets in Wnt signaling pathway 4

1.4.3 Newly identified Plekho1 as a novel molecular target 5

1.4.4 Osteogenic potential of *Plekho1* siRNA 6

1.5 Generation of targeted delivery system for osteogenic siRNA at tissue level 7

1.6 Application of aptamers 9

1.6.1 Advantages of aptamers 9

1.6.2 Selection of aptamers 10

1.6.3 Aptamers as targeting ligands 11

1.7 LNPs as delivery carriers 11

1.8 Research hypothesis 12

Chapter 2 Screening and identification of osteoblast-specific aptamer 13

2.1 Introduction 13

2.2 Experimental design 13

2.3 Materials and methods 14

2.3.1 Cell culture 14

2.3.2 Random library and primers in cell-SELEX 16

2.3.3 Cell-SELEX procedures 17

2.3.4 Binding assay in cell-SELEX 18

2.3.5 Statistical analysis 18

2.4 Results and discussion 19

2.4.1 Screening and identification of candidate aptamers 19

2.4.2 CH6 as the optimal aptamer after the modification 23

Chapter 3 *In vivo* organ/cell-specific delivery and gene knockdown 27

3.1 Introduction 27

3.2 Experimental design 28

3.2.1 *In vivo* organ-selective delivery of the osteogenic siRNA 28

3.2.2 *In vivo* cell-selective delivery of the osteogenic siRNA 29

3.2.3 *In vivo* cell-selective gene knockdown 30

3.3 Materials and methods 31

3.3.1 Animal handing 31

3.3.2 Biophotonic imaging analysis of the tissue distribution of siRNA 31

3.3.3 Analysis of tissue fluorescence by a microplate reader system 31

3.3.4 *In vivo* localization of Cy3-labeled siRNA in livers 32

3.3.4 *In vivo* Cy3-labeled siRNA cellular uptake in PBMCs..................32

3.3.5 Immunohistochemistry 32

3.3.6 Fluorescence-activated cell sorting (FACS) 33

3.3.7 Laser capture microdissection (LCM) 34

3.3.8 Real-time PCR 35

3.3.9 Statistical analysis 35

3.4 Results and discussion 36

3.4.1 *In vivo* bone-selective delivery of the osteogenic siRNA..............36

3.4.2 *In vivo* osteoblast-specific delivery of the siRNA 38

3.4.3 Accumulation of the siRNA in liver and MPS...............................42

3.4.4 Cellular uptake of the siRNA by PBMCs 43

3.4.5 *In vivo* osteoblast-selective gene knockdown 44

Chapter 4 *In vivo* dose response relationship and persistence of gene silencing...49

4.1 Introduction..49

4.2 Experimental design 49

4.2.1 *In vivo* dose-response pattern..49

4.2.2 *In vivo* persistence of gene silencing 50

4.3 Materials and methods 50
4.3.1 Fluorescence-activated cell sorting (FACS) 50
4.3.2 Real-time PCR 51
4.3.3 Statistical analysis 52
4.4 Results and discussion 52
4.4.1 Analysis of dose response relationship of siRNA formulations 52
4.4.2 Analysis of persistence of gene silencing of siRNA formulations..53
Chapter 5 Analysis of bone anabolic action in osteopenic rats..............................55
5.1 Introduction..55
5.2 Experimental design 55
5.3 Materials and methods 56
5.3.1 Animal handing 56
5.3.2 microCT analysis 57
5.3.3 Bone histomorphometry 58
5.3.4 Mechanical testing 59
5.3.5 ELISA assays of bone turnover markers.......................................59
5.3.6 Statistical Analysis 60
5.4 Results and discussion 61
5.4.1 microCT analysis to determine bone mass and micro-architecture 61
5.4.2 Bone histomorphometric analysis to determine bone formation 65
5.4.3 A three-point bending test to determine bone mechanical strength 67
5.4.4 ELISA assay to determine bone turnover markers 69

5.5.5 microCT analysis for cortical bone parameters 70
5.5.6 Trabecular bone parameters of the 5th vertebrae determined by microCT 72
Chapter 6 Comparison of bone anabolic action between the two siRNA delivery systems 74
6.1 Introduction..74
6.2 Experimental design 74
6.3 Materials and methods 75
6.3.1 microCT analysis 75
6.3.2 ELISA assays of bone turnover markers...75
6.3.3 Statistical analysis 76
6.4 Results and discussion 76
6.4.1 microCT analysis in healthy rats...76
6.4.2 ELISA assay to determine bone turnover markers in healthy rats..78
Chapter 7 *In vivo* toxicity and immune response of CH6-LNPs-siRNA 80
7.1 Introduction..80
7.2 experimental design 80
7.3 Materials and methods 81
7.4 Results and discussion 81
Chapter 8 Comparison of methods for generating ssDNA in SELEX...................84
8.1 Introduction..84
8.2 Experimental design 85

8.3 Materials and methods 86

8.3.1 Chemical reagents 86

8.3.2 ssDNA library and primers 86

8.3.3 PCR reaction 87

8.3.4 Agarose gel electrophoresis 88

8.3.5 Magnetic separation with streptavidin-coated beads 88

8.3.6 Separation of ssDNA by size separation derived from unequal primers 89

8.3.7 Statistical Analysis 89

8.4 Results and discussion 90

8.4.1 Technological process for generation of ssDNA 90

8.4.2 Optimization of PCR condition 92

8.4.3 Purity of ssDNA after the separation 96

8.4.4 Recovery rate of ssDNA 98

Chapter 9 Discussion 100

Reference 105

CURRICULUM VITAE 118