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Invited Review

Bone site-specific delivery of siRNA

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Abstract

Small interfering RNAs (siRNA) have enormous potential as therapeutics to target and treat various bone disorders such as osteoporosis and cancer bone metastases. However, effective and specific delivery of siRNA therapeutics to bone and bone-specific cells *in vivo* is very challenging. To realize the full therapeutic potential of siRNA in treating bone disorders, a safe and efficient, tissue- and cell-specific delivery system must be developed. This review focuses on recent advances in bone site-specific delivery of siRNA at the tissue or cellular level. Bone-targeted nanoparticulate siRNA carriers and various bone-targeted moieties such as bisphosphonates, oligopeptides $(Asp)_8$ and $(AspSerSer)_6$, and aptamers are highlighted. Incorporation of these bone-seeking targeting moieties into siRNA carriers allows for recognition of different sub-tissue functional domains of bone and also specific cell types residing in bone tissue. It also provides a means for bone-formation surface-, bone-resorption surface-, or osteoblast-specific targeting and transportation of siRNA therapeutics. The discussion mainly focuses on systemic and local bone-specific delivery of siRNA in osteoporosis and bone metastasis preclinical models.

Keywords: siRNA delivery, bone-specific delivery, bone-seeking nanoparticles, bone-targeting moiety, cancer bone metastasis, osteoporosis

Introduction

Bone is a mineralized connective tissue that functions as a structural framework for the body. Bone is composed of proteins and minerals. About 35% of the dry weight of mature bone is organic in nature, mostly collagen; the remainder is a complex inorganic calcium phosphate system of mainly hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$[1]. Bone is a dynamic living tissue that is composed of three different types of cells: osteoblasts, osteoclasts, and osteocytes. Osteoblasts are bone-forming cells and are derived from bone marrow stromal cells[2]. They produce dense, cross-linked collagen, and specialized proteins, which comprise the organic matrix of bone[3]. Osteoclasts are large, multinucleated bone-resorbing cells that arise from monocyte-macrophage lineage[2]. Osteoclasts secrete hydrogen ions to dissolve the mineral component of bone matrix and lytic enzyme, cathepsin K, to digest the bone matrix (mainly type I collagen)[4-5]. Osteocytes are star-shaped cells that represent terminally differentiated osteoblasts and are commonly found in mature bone; they can permeate the mineralized bone matrix. Bone is remodeled continuously during adulthood through the resorption of old bone by osteoclasts and the subsequent formation of new bone by osteoblasts.

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A number of bone diseases, such as osteoporosis and cancer bone metastases, are major public health problems. After age 40, bone destruction begins to exceed bone formation, leading to local or systemic reduction of bone mass, a disease state known as osteoporosis. For individuals with osteoporosis, bone fractures represent a life-threatening event. For every 10% of bone that is lost, the risk of fracture doubles[2]. Bone is also the third most common location for cancer metastasis, after the lung and liver. Bone metastasis occurs in approximately 70% of patients with advanced breast or prostate cancer[6-7]. Bone metastases leading to excess bone loss are generally classified as osteolytic (bone destructive). Those leading to bone deposition are considered as osteoblastic (bone forming). Both bone degradation and deposition likely occur early in the metastatic process[8]. Once tumors metastasize to bone, they are usually incurable[7].

Our increasing molecular understanding of disease-causing or disease-promoting genes of bone pathology has led to discovery of many new molecular targets for the prevention and treatment of osteoporosis and bone metastasis. RNA interference (RNAi) has enormous potential as a class of biologic therapy to reversibly silence any abnormal genes, especially for gene targets traditionally considered "undruggable" by small molecules. Continued advances in nanotechnology and novel biomaterials have provided opportunities and the tools to harness the therapeutic potential of RNAi for treatment of skeletal complications of osteoporosis and bone metastasis.

This review focuses on recent advances in improving bone-specific delivery of RNAi at a tissue or cellular level. The bone-targeted nanoparticulate drug delivery systems and various ligand-directed bone-targeted moieties such as bisphosphonates, oligopeptides $(Asp)_8$ and $(AspSerSer)_6$, and aptamers are highlighted. Both the systemic and local delivery of bone-targeted RNAi therapeutics in two kinds of bone disorders - osteoporosis and bone metastasis are described.

Delivery of siRNA therapeutics

Mechanisms of gene silencing by siRNA

Since the discovery of the RNAi mechanism in *Caenorhabditis elegans* by Andrew Fire, Craig Mello, and colleagues in 1998[9], RNAi has evolved as a new class of promising biological therapeutics for various diseases at a very rapid pace. RNAi therapeutics represent a fundamentally new way to treat human disease and target otherwise “undruggable” therapeutic targets. RNAi harnesses highly sequence-specific gene-silencing capabilities and has the potential to silence nearly any undesirable gene found in the body; thus it has enormous therapeutic potential in a broad range of diseases including various bone disorders[10].

The goal of RNAi-based therapy is to use sequence-specific small interfering RNAs (siRNA) to target and cleave complementary messenger RNA (mRNA) for efficient gene silencing[11]. The mechanism of RNAi is summarized in ***Fig. 1***. RNAi is triggered by the presence of long pieces of double-stranded RNA (dsRNA), which are cleaved into the fragments known as siRNA (21-22 nucleotides long with 2-nucleotides overhangs on the 3′ ends) by the endoribonuclease Dicer[12]. siRNA can be synthetically produced to mimic the Dicer cleavage product and then directly introduced into cytoplasm of the cell, where it is incorporated into a protein complex called the RNA-induced silencing complex (RISC). The duplex siRNA are then unwound; a guide strand is retained within the RISC, while the other passenger strand undergoes degradation by nucleases. The activated RISC-guide-strand complex identifies and cleaves mRNA that is exactly complementary to the guide strand, preventing translation and selectively silencing gene expression[13,14].

Challenges of siRNA delivery to bone

Effective and specific delivery of siRNA to the desired tissues and cells of interest efficiently and specifically *in vivo* is very challenging. siRNA molecules have unfavorable physicochemical properties such as large molecular weight and size, negative charges, and instability. The major limitations of delivery siRNA *in vivo* include premature nuclease degradation, reticuloendothelial system (RES) clearance, insufficient accumulation at organ or cells of interest, limited tissue penetration and cellular internalization, endosomal escape, and off-target effects. Several excellent reviews have outlined the physical, biological, and immunological barriers to siRNA delivery[15-16]. Successful siRNA delivery requires the use of a delivery vehicle, because unmodified naked siRNA is unstable in blood circulation and extracellular space. Direct systemic administration of naked siRNA in rats led to a very short half-life (6 minutes) due to degradation by serum nucleases[17]. To provide protection from endonucleases and overcome the short circulation problem of siRNA delivery, investigators designed many carrier systems such as lipid-based nanocarriers (liposomes, lipid nanoparticles), polymer-based systems (polyethylenimine, dendrimer, chitosan, collagen), and inorganic-based system (calcium phosphates) to entrap siRNAas cargo inside nanoparticles. Several

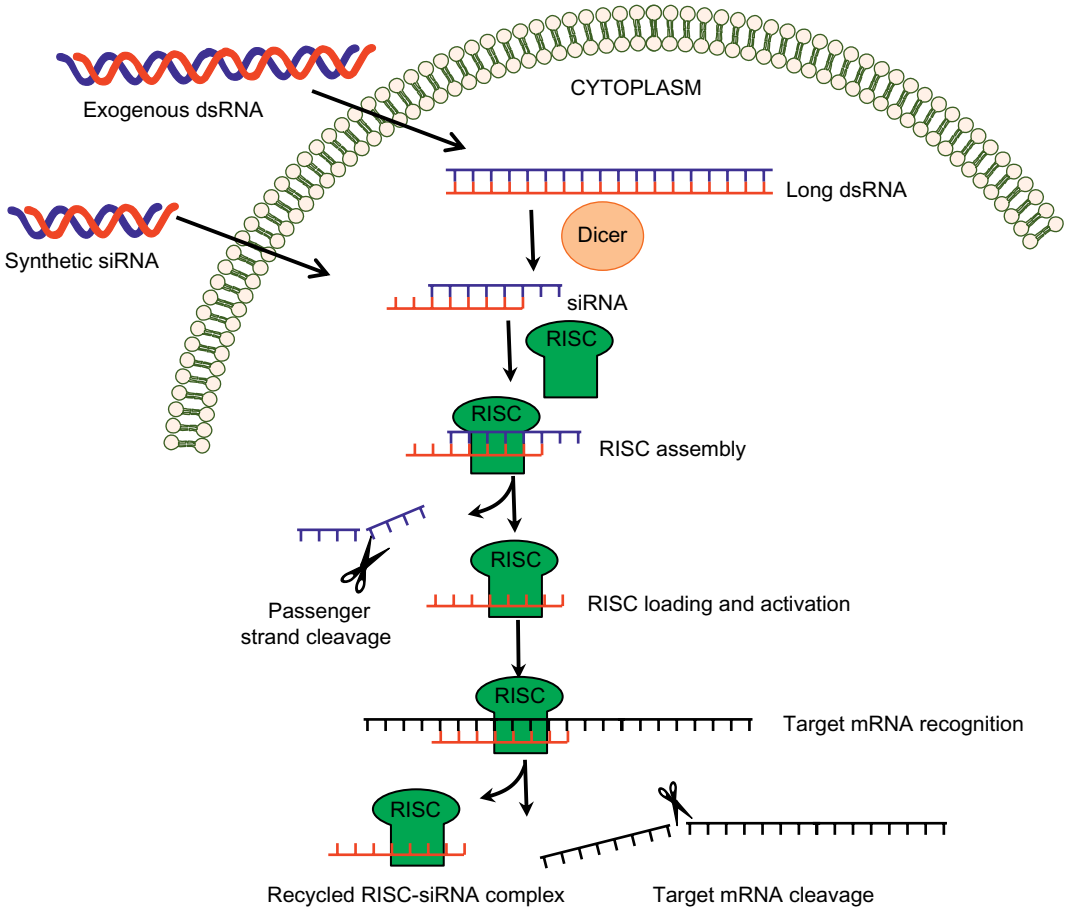

***Fig. 1* The RNAi pathway for post–transcriptional gene silencing.** When long double-stranded RNA (dsRNA) is introduced into the cytoplasm of a cell, the dsRNA is cleaved by the enzyme Dicer into siRNA (21-22 nucleotides long with 2-nucleotides overhangs on the 3′ ends). Alternatively, chemically synthesized siRNA can be transfected into cells to mimic that of Dicer products. The siRNA is then incorporated into the RNA-induced silencing complex (RISC), which can distinguish between the different strands of the siRNA. The RISC unwinds the siRNA duplex, the passenger strand (blue) is degraded, and the guide strand (red) is retained within the RISC. The activated RISC-guided strand complex binds to mRNA (black) through complementary base pairing, ultimately degrades the mRNA, and results in silencing of the target gene.

excellent publications offer very comprehensive reviews on various nanoparticulate delivery systems for *in vivo* application of siRNA therapeutics[14,18-21].

Delivery of siRNA to bone is even more challenging due to limited drug penetration in bone and poor vascular perfusion of bone tissue. Advancements in molecular biology towards understanding bone disorders have identified multiple therapeutic pathways that can be potentially intervened by siRNA. However, many molecular targets identified are osteoblast- or osteoclast-specific; cell-specific delivery of siRNA to osteoblasts or osteoclasts selectively *in vivo* is still lacking. Targeted delivery of siRNA with high affinity for bone or bone-specific cells is critical for avoiding off-target and unwanted effects and subsequent toxicity to the body. Previous small molecule studies have demonstrated the importance of drug delivery to desired organs and tissues. For instance, in osteoporosis treatment with estrogen, the distribution of estrogen to other tissues besides bone can cause several severe side effects such as intrauterine hemorrhage and occasionally endometrial and breast cancer[22]. Recent innovations in materials science and nanotechnology bring the possibility of selecting among a wide range of biomaterials as carriers for siRNA delivery to bone. These carriers coupled with bone-homing molecules increase binding avidity to bone and decrease off-target effects in other organs. This review focuses on these bone-targeted molecules and carrier systems in the preclinical models of osteoporosis and bone metastasis.

Systemic delivery of siRNA to bone

Systemic delivery of siRNA to bone is not very efficient because bones are not a highly perfused tissue. Intravenous administration of nanocarrier-contained siRNA usually results in substantial accumulation in both liver and spleen as a result of immune clearance

by the RES. Efforts have been made to modify nanocarriers with bone-seeking targeting moieties to enhance their localization at bone and improve siRNA delivery in a restricted cell population *in vivo*. To date, most researchers rely on bisphosphonates as a bone-targeting moiety. Bisphosphonates, such as alendronate, target both bone-formation surfaces and bone-resorption surfaces. The $(Asp)_8$ and $(AspSerSer)_6$ oligopeptides provide molecular recognition of the bone-resorption surface and bone-formation surface, respectively[23-24]. Recently, aptamer CH6 was identified as an osteoblast-specific molecule[25] (***Table 1***). This section reviews different bone-seeking targeting ligands that can guide siRNA and its carriers to bone or bone-specific cells.

Atelocollagen-mediated siRNA delivery

Bone matrix proteins are mainly collagen, a natural biocompatible polymer with three polypeptide chains forming a helix. Atelocollagen is a highly purified pepsin-treated type I collagen of calf dermis. Under physiological conditions, atelocollagen forms a fiber-like natural collagen. It is low in immunogenicity and toxicity and used widely in the clinic for wound healing and as bone cartilage substitute[26]. Atelocollagen has been used as a carrier for plasmid DNA for controlled gene transfer[26-27].

The atelocollagen-mediated delivery system was used to target siRNA to bone-metastatic lesions to silence endogenous genes involved in skeletal metastasis of prostate cancer[28]. The siRNA/atelocollagen complex is resistant to nuclease and can be efficiently transduced into cells, allowing for long-term gene silencing. Intravenous injection of GL3 luciferase siRNA/atelocollagen complex showed effective reduction of luciferase expression from bone-metastatic prostate tumor cells developed in mouse thorax, jaws, and legs. The siRNA/atelocollagen complex can be efficiently delivered to bone-metastatic tumors 24 hours after injection and existed intact for 3 days. When using therapeutic siRNAs, such as enhancer of zeste homolog 2 (*EZH2*) and phosphoinositide 3′-hydroxykinase p110-alfa-subunit (*p110-α*), the systemic administration of *EZH2* and *p110-α* siRNA/atelocollagen complexes in a mouse model of bone metastasis demonstrated effective gene silencing and efficient inhibition of metastatic tumor growth in bone tissues. siRNA/atelocollagen complexes showed greater selective accumulation in bone-metastatic tumors, compared with normal tissues. This could be due to the prolonged circulation time of high-molecular-weight macromolecules and enhanced permeability and retention (EPR) effect[28]. Atelocollagen is a useful biomaterial to mediate siRNA delivery to bone metastasis.

Bisphosphonate as a bone-targeting moiety

Bisphosphonates (BP) are very well-known drugs that prevent the loss of bone mass in osteoporotic patients. BP have a high affinity for hydroxyapatite, the main mineral component of bone, through Ca^{2+} ion-mediated coordination bonding. BP have been utilized as a bone-targeting group and have been conjugated to drug molecules, imaging agents, proteins, polymers, and nanoparticles to provide specific bone-targeting capability[29-31].

Bisphosphonate-conjugated calcium phosphate nanoparticles were used as an efficient carrier for transporting siRNA[32]. In this study, alendronate (ALN), a nitrogen-containing BP, was conjugated to polyethylene glycol (PEG); the PEG-ALN was used as a PEGylated-chelating agent to stabilize calcium phosphate nanoparticles. Specifically, $CaCl_2$ solution was added to Na_2HPO_4 solution followed by immediate addition of ALN. siRNA was pre-dissolved in $CaCl_2$ solution and thus became entrapped in the formed calcium-phosphate nanoparticles. The BP-stabilized nanoparticles efficiently delivered siRNA to cell lines and induced gene-silencing[32,33]. Although the nanoparticles was not tested *in vivo*, the preferred bone-targeting is expected due to BP's ability to bind to hydroxyapatite[29-31,34].

$(Asp)_8$ oligopeptide-guided bone delivery

Bone-formation surfaces, which are covered with osteoblasts, are mainly comprised of lowly crystallized hydroxyapatite and amorphous calcium phosphates. In contrast, the bone-resorption surfaces, which are covered with osteoclasts, are characterized by highly crystallized

Table 1 **Bone-seeking targeting moieties**

Bone-targeting moiety	Targeting sites and Mechanisms	References
Bisphosphonates	Bind to both the bone-formation and bone-resorption surfaces. Strong affinity to hydroxyapatite via calcium ion-mediated coordination bonding.	[29-31]
$(Asp)_8$ or $(Asp)_6$ oligopeptide	Preferentially binds to highly crystalized hydroxyapatite on the bone-resorption surface.	[24,37]
$(AspSerSer)_6$ oligopeptide	High affinity for lowly crystalized hydroxyapatite and calcium phosphate on the bone-formation surface.	[23,38]
CH6 aptamer	Binds specifically to osteoblasts at the cellular level.	[25]

hydroxyapatite[24]. Kopecek′s group identified a D-aspartic acid octapeptide $(Asp)_8$ as a bone-targeting moiety that could recognize resorption sites in skeletal tissues, especially in bone sites with high turnover, such as the tibia and femur heads, lumbar vertebrae, and mandibular bone[24]. While another bone-targeting bisphosphonate moiety, alendronate, usually binds to both bone formation and bone-resorption sites[24]. Using atomic force microscopy, Kopecek and coworkers demonstrated that alendronate has a stronger binding force to hydroxyapatite than $(Asp)_8$, and $(Asp)_8$ is more sensitive to changes in hydroxyapatite crystallinity than is alendronate. They reasoned that it was the weak binding ability of $(Asp)_8$ to hydroxyapatite that caused its *in vivo* selectivity to the bone resorption surface (containing bone apatite with relatively higher crystallinity) over formation surfaces (mainly amorphous calcium phosphate)[24].

Zhang *et al*. fabricated a bone-targeting D-Asp_8-HPMA (*N*-(2-hydroxypropyl)methacrylamide copolymer) polymeric nanoparticle to deliver siRNA molecules to interfere with *Semaphorin 4D* (*sema4D*) expression[35]. Previous study demonstrated that incorporation of $(Asp)_8$ as a bone-targeting moiety could favorably deposit HPMA copolymers onto the entire skeleton, in particular, to high bone turnover sites[36]. *Sema4D* is a molecule found in an osteoporotic phenotype and plays a key role in osteoclast activity by suppressing osteoblast maturation, thus significantly altering the bone-modeling cycle. The authors demonstrated that the intracellular trafficking of siRNA within osteoclasts achieved knockdown of *sema4D* by more than 80% in osteoclasts that derived from both healthy and ovariectomy-induced osteoporotic mice. Intravenously administered rhodamine-labeled D-Asp_8-HPMA nanoparticle was thoroughly and evenly distributed to the entire skeleton, increasing its bone-targeting more than three-fold when compared with controls. Weekly intravenous administration of D-Asp_8-HPMA/*sema4D* siRNA led to a significantly greater number of active osteoblasts at the bone surface, resulting in higher bone volume in an ovariectomy-induced osteoporosis animal model. Furthermore, more than a four-fold decrease in the expression of *sema4D* was observed in osteoclasts after injection compared to control osteoclasts without the drug-delivery system. The site-specific delivery of the siRNA targeting *sema4D* to the bone resorption surface indirectly induced osteoclast maturation, subsequently reversing the osteoporotic phenotype[35].

In addition to the (D-Asp_8) octapeptide, *L*-aspartic acid hexapeptide (L-Asp_6) was also reported to have high affinity for hydroxyapatite and was used as a bone targeting ligand for the treatment of bone diseases such as osteoporosis and osteomyelitis[37].

$(AspSerSer)_6$ oligopeptide-guided delivery

Bone-formation surfaces have lowly crystalized hydroxyapatite as well as calcium phosphate whereas bone resportion surfaces have highly crystalized hydro xyapatite[24]. The six repeating sequences of the tripeptide aspartate-serine-serine $(AspSerSer)_6$ motif bind to the small and randomly oriented hydroxyapatite crystals that are a feature of *de novo* bone surfaces. Additionally, the $(AspSerSer)_6$ peptide also showed favorable binding to osteoblast-mediated mineralizing nodules and amorphous calcium phosphate *in vitro*. All of these features make the $(AspSerSer)_6$ peptide a selectively targeting moiety for the bone-formation surface and not the bone-resorption surface[23,38].

By taking advantage of the $(AspSerSer)_6$ peptide's propensity to bind to lowly crystalized hydroxyapatite and amorphous calcium phosphate in osteoblast-covered bone-formation surfaces, Zhang's group developed a targeting system involving dioleoyl trimethylammonium propane (DOTAP)-based cationic liposomes attached to $(AspSerSer)_6$ for delivering osteogenic siRNA *Plekho1* to osteogenic-lineage cells[23]. The osteogenic *Plekho1* siRNA targets casein kinase-2 interacting protein-1, a suppressor of bone formation. The inhibitory gene *Plekho1* is expressed in bone-lining cells and active osteoblasts. Silencing this gene can block a negative regulator of bone formation and, therefore, potentially promote bone formation, making it a potentially useful therapeutic for osteoporosis. Specific delivery of this therapeutic siRNA into osteogenic-lineage cells, not to other cells, is critical to realizing its therapeutic potential. $(AspSerSer)_6$-functionalized nanoparticles allows for specific delivery of osteogenic siRNAs to osteoblast-enriched bone-forming surfaces to silence bone-formation-inhibitory genes without affecting bone resorption[23].

Zhang and colleagues first encapsulated the *Plekho1* siRNA in a DOTAP cationic liposomes delivery system. The liposomes surface was covalently linked to the $(AspSerSer)_6$ peptide moiety to preferentially anchor and target the bone formation surface. Systemic delivery of fluorescently-labeled $(AspSerSer)_6$-liposomes in rats led to strong fluorescence signal in intrabecular bone but little activity in other organs. In contrast, DOTAP liposomes without the targeting peptide had a weaker fluorescence signal in intrabecular bone and more non-specific fluorescence in RES-associated organs, such as the liver. Moreover, systemic delivery of

$(AspSerSer)_6$-liposome-*Plekho1* siRNA in rats led to enrichment of the siRNAs in osteogenic cells, effectively silenced *Plekho1* in bone, and resulted in nearly two-fold increase in bone formation and no changes in bone resorption in rats. The $(AspSerSer)_6$-liposomes delivery system targeted the therapeutic siRNA specifically into osteogenic-lineage cells, not other cells in rats and, therefore, promoted bone formation without negatively affecting bone resorption[23].

Aptamer-guided bone delivery

Aptamers are a class of small (25-35 bases long), single-stranded RNA (ssRNA) or DNA (ssDNA) nucleic acids sequences that, when folded into their unique tertiary conformation, can recognize and bind to their targets with high specificity and affinity[39]. Aptamers are identified from a combinatorial library of randomized sequences through repeated rounds of selection, known as "systemic evolution of ligands by exponential enrichment (SELEX)"[40,41]. The targets of aptamers can be small molecules, nucleic acids, proteins, carbohydrates, and whole cells. Aptamers generally achieve the same affinities and specificities as antibodies[42]. siRNA can be conjugated to aptamer directly as aptamer-siRNA chimeras[43] or encapsulated in aptamer-functionalized nanoparticles for delivery to specific cell types[44].

To further decrease the potentially harmful off-target effects, Zhang's group developed an improved aptamer-functionalized lipid nanoparticles (LNP) as a true osteoblast-specific delivery system[25]. They first screened a library of ssDNA molecules using a cell-based SELEX technique to select osteoblast-specific aptamers. Osteoblast-specific aptamer CH6 (5′-AGTCTGTTGGACCGAATCCCGTGGACGCACCCTTTGGACG-3′) was identified to have the ability to specifically bind both rat and human osteoblasts with high affinity, but not human osteoclasts or liver cells. The CH6 aptamer was modified with 2′-O-methyl-nucleotide substitutions to minimize nuclease degradation. Then they fabricated an aptamer CH6- functionalized LNP to achieve direct osteoblast-specific delivery of osteogenic siRNA for the gene *Plekho1*, a negative regulator of bone formation. The CH6-LNP-siRNA nanoparticles had a small size (84±5 nm) with high PEG shielding to avoid nonspecific RES uptake. The osteoblast-specific CH6-LNP-siRNA facilitated osteoblast-selective uptake of *Plekho1* siRNA *in vitro*, and boosted osteoblast-specific *Plekho1* gene silencing *in vivo* compared with nonfunctionalized LNP-siRNA or LNP functionalized with a random-sequence aptamer (Rd-LNP-siRNA). CH6-LNP-siRNA showed higher accumulation in bone tissue and lower accumulation in the liver and kidney in rats compared to LNP-siRNA and Rd-LNP-siRNA. The siRNA co-localized with osteoblast-specific markers, alkaline phosphatase and osteocalcin, but not with the osteoclast markers, osteoclast-associated receptor and cathepsin K, confirming the osteoblast-specificity of the CH6-LNP-siRNA formulation. Ovariectomized rats treated with CH6-LNP-siRNA had improved bone microarchitecture, increased bone mass, and enhanced mechanical properties without adverse effects. The study demonstrated that CH6 aptamer-functionalized LNP improved bone delivery at the tissue and cellular level[25].

Aptamer CH6-LNP-siRNA achieved better gene silencing and bone anabolic action when compared to $(AspSerSer)_6$-liposome-siRNA. The authors rationalized that the CH6-LNP-siRNA was more specific and efficient because CH6 aptamer induced macropinocytosis and facilitated siRNA entry in osteoblasts. The authors also attributed this therapeutic difference to the distinct mechanisms responsible for targeted delivery. The $(AspSerSer)_6$ moiety targets the physiochemical features of the bone formation surface (targeting at the tissue level), whereas CH6 aptamer adopts distinct tertiary structures to directly bind to target osteoblasts (targeting at the cellular level)[25].

Local delivery of siRNA to bone

In addition to the systemic administration of siRNA and its carrier, local delivery of siRNA directly to bone has also been explored. siRNA delivery system can be directly and specifically applied to different bone sites such as femur, vertebral spine, and wrist. In general, local-regional delivery of siRNA has fewer barriers compared to systemic delivery[15]. Local delivery can avoid or delay RES uptake, reduce undesirable systemic toxicity, and improve organ specificity[16]. Local delivery of siRNA into or around bone tissue can improve the concentration effectively reaching the bone; therefore, the dose needed may be substantially lower.

Manaka et al. reported a local delivery system utilizing a biodegrade hydrogel, poly-*D,L* lactic acid-*p*-ioxanonepolyethylene glycol block copolymer (PLA-DX-PEG) carrier, to deliver siRNA to silence *noggin*, an antagonist to bone morphogenetic protein-2 (BMP-2)[45]. The PLA-DX-PEG pellets with siRNA were locally implanted into mouse dorsal muscle pouches. The sustained local release of the *noggin* siRNA led to suppression of *noggin* gene expression and promotion of new bone formation in mice[45].

Grainger and coworkers described a passive phagocyte-targeted local delivery system to target *RANK* siRNA to bone sites to down-regulate osteoclast formation and function[46]. *RANK* plays a key role in regulating osteoclastogensis. Activation of *RANK* by its ligand, *RANKL*, is required for the formation and activation of osteoclasts. The authors prepared poly(lactic-co-glycolic acid) (PLGA) microspheres (size = 5 μm, zeta potential = –21.07 mV), and then encapsulated *RANK* siRNA/bPEI polyplex into PLGA microparticles using the double-emulsion method. The bPEI (branched polyethyleneimine, 25 kDa) is a commonly used non-viral nucleic acid delivery vector and can increase the stability and loading efficiency of siRNA in PLGA microparticles. The PLGA microparticles were used as a passive phagocyte-targeted carrier to deliver siRNA to both osteoclast precursors and osteoclasts - the professional phagocytes in bone. The authors hypothesized that the natural phagocytes internalize micron-sized PLGA particles while most other non-targeted cells in bone cannot. They showed that the *RANK* siRNA/bPEI-PLGA microparticles effectively reduced *RANK* expression in bone marrow cell cultures compared with control siRNA/bPEI-PLGA microparticles. PLGA-siRNA microparticles were dispersed within biomedical grade calcium phosphate cement (CPC), clinically used in osteoporosis as a bone augmentation biomaterials for fragility fracture prevention and fixation. The authors demonstrated that *RANK* siRNA-loaded PLGA/CPC led to significant suppression of *RANK* expression in murine bone marrow cells seeded on CPC wafer. The results support the concept of applying the unique combination of injectable bone-augmenting materials and local siRNA delivery to bone sites for the treatment of osteoporosis[46].

Conclusion remarks

Osteotropic drug delivery system of RNAi is still in its infancy. Recent advances in nanotechnology have enhanced target-specificity to bone and provided a means to modulate biodistribution of siRNA at both the tissue and cellular levels. Incorporation of molecular structures such as bisphosphonate, $(Asp)_8$ and $(AspSerSer)_6$ oligopeptides allows for recognition of different sub-tissue functional domains of bone and provides a means for bone site-specific targeting and transportation of siRNA therapeutics. Osteoblast-specific and nuclease-resistant aptamer targeting moiety facilitates osteoblast-specific delivery of siRNA while avoiding potential toxic effects in other cells such as endothelial cells and lymphocytes in bone microenvironment. It is anticipated that more bone-targeted ligands capable of specific binding to disease biomarkers will be identified in future. This will play an important role in designing new drug and bone-targeted drug delivery systems, which will greatly enhance the therapeutic index of RNAi therapeutics by delivering them directly to the desired cells and molecular targets. With the rapid discovery of new and critical regulatory genes for various bone disorders, successful application of a safe and specific RNAi delivery system for efficient silencing of disease-causing genes will positively impact the prevention and treatment of diseases of the bone.

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