Notice

DESCRIPTION CN116024210A

An osteoporosis biomarker circRNA and its application

一种骨质疏松标志物circRNA及其应用

[0001]

Technical Field

技术领域

[n0001]

This invention relates to the field of biotechnology, and in particular to an osteoporosis biomarker circRNA and its applications.

本发明涉及生物技术领域，特别是涉及一种骨质疏松标志物circRNA及其应用。

[0003]

Background Technology

背景技术

[n0002]

Overactivation of multinucleated cells plays an important role in the pathological process of many diseases. Osteoclasts (OCs) are typical multinucleated cells that differentiate from monocyte/macrophage lineages by stimulating macrophage colony-stimulating factor (M-CSF) and nuclear factor-κB ligand receptor activator.

多核细胞的过度活化在许多疾病的病理过程中起着重要的作用，破骨细胞(OCs)是典型的多核细胞，通过刺激巨噬细胞集落刺激因子(M-CSF)和核因子-κB配体受体激活物从单核/巨噬细胞谱系分化而来。

Multinucleated cells have high transcriptional activity, while mature multinucleated osteoclasts transform into the main bone resorption cells through transcription, synthesis and secretion of large amounts of enzymes and acids.

多核细胞具有较高的转录活性，而成熟的多核破骨细胞则通过转录、合成与分泌大量的酶和酸，转化为最主要的骨吸收细胞。

However, excessive osteoclast polymorphism ultimately leads to an imbalance in bone remodeling, resulting in osteolytic diseases such as osteoporosis.

然而，过度的破骨细胞多核化最终导致骨重建失衡，导致骨质疏松等溶骨性疾病。

Therefore, understanding the regulatory process of osteoclast multinucleation is key to studying the pathogenesis and regulatory mechanisms of osteoporosis.

因此，围绕着破骨细胞多核化的调控过程，是研究骨质疏松发病过程及调控机制的关键。

[n0003]

Currently, first-line treatments for osteolytic diseases, such as bisphosphonates, can significantly inhibit the osteoclast lineage, leading to apoptosis of all bone resorbing cells, inhibiting necessary bone turnover, and thus causing atypical femoral fractures.

目前在溶骨性疾病的一线治疗，如双膦酸盐，能够明显抑制破骨细胞谱系，导致所有骨吸收细胞凋亡，抑制了必要的骨转换，进而引发非典型股骨骨折。

As our understanding of osteoclast differentiation deepens, the classification of osteoclast lineages has become increasingly clear. The RANKL-induced osteoclast differentiation process includes the assembly of monocyte precursors and membrane rafts, the initiation of RNA metabolism, the terminal differentiation of pre-osteoclasts (pOCs), and finally their fusion with mOCs. Compared to mOCs, pOCs do not undergo multinucleation, but they can retain a certain level of bone resorption activity, maintain positive communication with osteoblasts, and maintain normal osteoogenesis. Therefore, developing a spatiotemporally selective strategy to inhibit osteoclast lineages in pOCs and prevent their multinucleation process can avoid the shortcomings of current clinical drugs that "don't differentiate lineages and treat all cases the same".

随着对破骨细胞分化认识的深入加深，破骨细胞谱系的划分已经越来越清晰。 RANKL所诱导的破骨细胞分化过程包括单核细胞前体、膜筏的组装、RNA代谢的启动、破骨细胞前体细胞(pre-

osteoclasts,pOCs)的终末分化，最后融合于mOCs的过程。 与mOCs相比， pOCs没有多核化，但能够保留一定水平的骨吸收活性，可与成骨细胞保持正向沟通，维持了正常的成骨。 因此，开发一种时空选择性策略，通过抑制pOCs中的破骨细胞谱系，阻止其多核化的进程，能够避免现用临床药物“不分谱系，一概而论”的缺陷。

[n0004]

Therefore, based on the above background, this patent discovers an osteoclast-specific circBBS9 and its human homology hsa_circ_0134188, which are specifically expressed in osteoclast multinucleation and osteoporosis; and through siRNA^{circBBS9}, it can effectively inhibit osteoclast multinucleation and bone resorption without affecting the differentiation of osteoclast precursor cells.

因此，基于上述背景，本专利发现一种破骨细胞特异性circBBS9及其人类同源hsa_circ_0134188，在破骨细胞多核化和骨质疏松过程中特异性表达；并且通过siRNA^{circBBS9}能够有效抑制破骨细胞的多核化和骨吸收能力而不影响破骨细胞前体细胞的分化。

Meanwhile, miR-423-3p functions as a downstream product of circBBS9. Corresponding in vivo experiments confirmed that siRNA designed for this circRNA can effectively increase bone density in osteoporotic mice. In summary, circBBS9 has the potential to serve as a biomarker for osteoporosis and can be used in clinical research on osteoporosis drugs.

同时，miR-423-3p作为circBBS9的下游发挥作用。 相应的体内实验证实针对此 circRNA设计的siRNA可有效增加骨质疏松小鼠的骨密度。 综上，circBBS9有作为骨质疏松标志物的潜力，可用于临床上骨质疏松的药物研究。

[0007]

Summary of the Invention

发明内容

[n0005]

In view of the shortcomings of the prior art, this invention proposes an osteoporosis biomarker circRNA and its application.

本发明针对现有技术的不足，本发明提出了一种骨质疏松标志物circRNA及其应用。

[n0006]

To achieve the above objectives, the present invention provides the following solution:

为实现上述目的，本发明提供了如下方案：

[n0007]

An osteoporosis biomarker circRNA, wherein the circRNA is circBBS9 and its human homolog, and its nucleotide sequence is shown in SEQ ID NO.1 and SEQ ID NO.2.

一种骨质疏松标志物circRNA，所述的circRNA为circBBS9及其人类同源物，其核苷酸序列如SEQ ID NO.1以及SEQ ID NO.2所示。

The upregulation of circBBS9 expression resulted in increased osteoclast multinucleation and bone resorption capacity.

所述的circBBS9表达上调造成破骨细胞多核化水平和骨吸收能力增强。

[n0008]

The application of circRNA, an osteoporosis biomarker, as a target for osteoporosis intervention, with miR-423-3p as its downstream target.

一种骨质疏松标志物circRNA作为骨质疏松干预靶点的应用，miR-423-3p为其下游靶点。。

[n0009]

The application of circRNA, an osteoporosis marker, as a drug for the prevention and treatment of osteoporosis, involves knocking down the expression level of circBBS9 to obtain siRNA. The nucleotide sequence of the siRNA is shown in SEQ ID NO.7 and SEQ ID NO.8. The application of siRNA in the preparation of drugs for the prevention and treatment of osteoporosis is also discussed.

一种骨质疏松标志物circRNA作为防治骨质疏松的药物的应用，敲减circBBS9表达量得到siRNA，所述的siRNA的核苷酸序列如SEQ ID NO.7和SEQ ID NO.8所示， siRNA在制备防治骨质疏松的药物中的应用。

[n0010]

The application of circRNA, an osteoporosis marker, as a drug for the prevention and treatment of osteoporosis, involves knocking down the expression level of circBBS9 to obtain siRNA. The nucleotide sequence of the siRNA is shown in SEQ ID NO.7 and SEQ ID NO.8. The application of the siRNA expression vector in the preparation of drugs for the prevention and treatment of osteoporosis is also discussed.

一种骨质疏松标志物circRNA作为防治骨质疏松的药物的应用，敲减circBBS9表达量得到siRNA，所述的siRNA的核苷酸序列如SEQ ID NO.7和SEQ ID NO.8所示， siRNA的表达载体在制备防治骨质疏松的药物中的应用。

[n0011]

As a preferred option, the expression vector for siRNA is biomimetic bone-targeting nanoparticles.

作为优选，siRNA的表达载体为仿生骨靶向纳米颗粒。

[n0012]

The beneficial effects of this invention are:

本发明的有益效果：

[n0013]

The osteoporosis marker circBBS9 of this invention is expressed at elevated levels in osteoporosis patients and mice, and exhibits a stage-specific expression form in osteoclasts. Furthermore, it can effectively inhibit osteoclast multinucleation and reduce bone resorption area through siRNA^{circBBS9}, while preserving the differentiation capacity of osteoclast precursor cells.

本发明的骨质疏松标志物circBBS9在骨质疏松患者和小鼠中表达量升高，且在破骨细胞中具有阶段特异性的表达形式，并且通过siRNA^{circBBS9}可以有效抑制破骨细胞多核化，减少骨吸收面积，同时保留破骨细胞前体细胞的分化能力。

It can achieve the effect of treating osteoporosis.

能够实现治疗骨质疏松的效果。

[0017]

Attached Figure Description

附图说明

[n0014]

Figure 1. Expression of circBBS9 in osteoporosis.

图1circBBS9在骨质疏松中的表达。

(a) Heatmap analysis of differentially expressed circRNAs in RNA-seq.

(a)RNA-seq中差异性表达的CircRNA热图分析。

(b) RT-qPCR showed that circBBS9 expression increased significantly after RANKL stimulation, with the most significant increase occurring during the third day of multinucleation.

(b)RT–qPCR表明circBBS9经RANKL刺激后表达显著上升，在第三天多核化阶段最为显著。

(c) Changes in circBBS9 expression during osteoclastization.

(c)破骨多核化中的circBBS9表达量变化。

[n0015]

Figure 2 shows the differential expression of has-circBBS9.

图2has-circBBS9的差异性表达。

(a) The human homolog of circBBS9 is expressed at elevated levels in the lumbar spine of patients with osteoporosis, suggesting it may be a biomarker for osteoporosis.

(a)circBBS9人类同源物在骨质疏松患者的腰椎中表达上升，考虑其是骨质疏松的标志物可能。

(b) The expression level of the human homolog circBBS9 in osteoclasts derived from human peripheral blood cells was significantly higher than that in macrophages.

(b)circBBS9人类同源物在人外周血细胞来源的破骨细胞中的表达量显著高于巨噬细胞。

[n0016]

Figure 3. Effects of siRNA^{circBBS9} transfection on marker gene expression at different differentiation stages of osteoclasts.

图3转染siRNA^{circBBS9}后对破骨细胞不同分化阶段的标记基因表达的影响。

[n0017]

Figure 4 shows that TRAP staining demonstrates that siRNA^{circBBS9} can inhibit multinucleation in osteoclasts.

图4TRAP染色证明siRNA^{circBBS9}可以抑制破骨细胞的多核化。

(a, b) Representative images of TRAP-positive cells 5 days after transfection with siRNA^{circBBS9} and co-culture with RANKL on the specified date. (c) Quantitative statistics of TRAP-positive cells.

(a，b)在指定日期用siRNA^{circBBS9}转染及RANKL共培养5天后，TRAP阳性细胞的代表性图像(c) TRAP阳性细胞的定量统计

[n0018]

Figure 5 shows bone resorption plates, demonstrating that siRNA^{circBBS9} reduces the bone resorption capacity of osteoclasts.

图5骨吸收板证明siRNA^{circBBS9}减少了破骨细胞的骨吸收能力。

(a) Representative images and quantification of bone slice absorption area 5 days after transfection with siRNA^{circBBS9} and co-culture with RANKL on the specified date.

(a)在指定日期用siRNA^{circBBS9}转染及RANKL共培养5天后，骨片吸收面积的代表性图像和定量。

Scale bar, 200μm.

比例尺，200μm。

(b) Quantitative statistics on bone fragment absorption area

(b)骨片吸收面积的定量统计

[n0019]

Figure 6 shows that siRNA^{circBBS9} inhibited osteoclast-characterizing proteins.

图6Western blot表明siRNA^{circBBS9}抑制了破骨细胞表征蛋白。

[n0020]

Figure 7 shows that siRNA^{circBBS9} inhibited the expression level of osteoclast characterization genes.

图7RT-qPCR表明siRNA^{circBBS9}抑制了破骨细胞表征基因的表达水平。

[n0021]

Figure 8 shows that TRAP staining of the surface miR-423-3p sponge reversed the process of osteoclast multinucleation induced by siRNA^{circBBS9}.

图8TRAP染色表面miR-423-3p sponge逆转了siRNA^{circBBS9}对破骨细胞多核化的进程。

[n0022]

Figure 9 shows that siRNA^{circBBS9} inhibits osteoclast multinucleation in vivo. By analyzing H&E and TRAP staining images of patients in each group and quantitatively calculating the trabecular volume ratio (BV/TV), osteoclast number (OC.N/BS), and osteoclast area (OC.S/BS), it was found that mice in the siRNA^{circBBS9} group exhibited fewer osteoclasts.

图9siRNA^{circBBS9}在体内抑制破骨细胞的多核化，通过对各组患者的H&E和TRAP 染色图像予以分析并对骨小梁体积比(BV/TV)、破骨细胞数量(OC.N/BS)、破骨细胞面积(OC.S/BS)值进行定量统计，发现siRNA^{circBBS9}组小鼠展现出更少的破骨细胞。

[n0023]

Figure 10 shows that siRNA^{circBBS9} can effectively reduce bone loss in osteoporotic mice.

图10siRNA^{circBBS9}能有效减少骨质疏松小鼠的骨质流失。

(a) Typical images of sections of the femur and tibia.

(a)股骨和胫骨切片的典型图像。

(b-e) Quantitative measurements of bone microstructure parameters were performed in each group, including trabecular volume ratio (BV/TV), number of trabeculae (TB.N), trabecular thickness (Tb.Th), and intertrabecular spacing (Tb.Sp).

(b-e)各组定量测量骨显微结构相关参数，包括骨小梁体积比(BV/TV)，骨小梁数目(TB.N)，骨小梁厚度(Tb.Th)和骨小梁之间的间隙(Tb.Sp)。

[0028]

Detailed implementation method:

具体实施方式：

[n0024]

The following detailed description, in conjunction with embodiments, illustrates an osteoporosis biomarker circRNA and its applications provided by the present invention, but these descriptions should not be construed as limiting the scope of protection of the present invention.

下面结合实施例对本发明提供的一种骨质疏松标志物circRNA及其应用进行详细的说明，但是不能把它们理解为对本发明保护范围的限定。

[n0025]

Example 1: Analysis of circBBS9 expression in osteoclasts

实施例1circBBS9在破骨细胞中的表达情况分析

[n0026]

1. This invention uses RNA-seq to detect differentially expressed circRNAs during osteoclast multinucleation.

1、本发明采用RNA-seq对破骨细胞多核化过程中差异表达的circRNA进行检测。

[n0027]

2. Design specific primers based on the obtained circRNA, and use PCR to verify the expression levels of these primers.

2、根据得到的circRNA设计特异性引物，使用PCR分别验证这些引物的表达量。

The results showed that circBBS9 expression level increased significantly after RANKL stimulation.

结果发现，circBBS9表达量在RANKL刺激后显著上升.

[n0028]

3. The expression level of circBBS9 in osteoporotic mice and osteoclast differentiation process was analyzed by PCR. It was found that the expression level was most significantly upregulated on the third day after RANKL stimulation (the osteoclast multinucleation stage), as shown in Figure 1.

3、采用PCR对circBBS9在骨质疏松小鼠和破骨细胞分化进程中的表达量予以分析，发现给以RANKL刺激后第三天(破骨细胞多核化阶段)表达量上调趋势最为明显，如图1。

The sequence of CircBBS9 is shown below (SEQ ID NO.1), and its PCR primers are shown as (SEQ ID NO.2) and (SEQ ID NO.3).

CircBBS9的序列如下所(SEQ ID NO.1)示，其PCR引物如(SEQ ID NO.2)和(SEQ ID NO.3)所示。

[n0029]

mmu_circ_0001757(SEQ ID NO.1)

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mmu_circ_0001757(SEQ ID NO.1)

[n0030]

GTGGCTGTACTCCAATCCCAGAGTCAGACCTAGAGGAAAGGTCACTAGATGACTCCACAGAGCTGTTTAC

GTGGCTGTACTCCAATCCCAGAGTCAGACCTAGAGGAAAGGTCACTAGATGACTCCACAGAGCTGTTTAC

[n0031]

primer-circBBS9-F(SEQ ID NO.2)

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primer-circBBS9-F(SEQ ID NO.2)

[n0032]

TGGAGTAATGCTAATGAGTTGAGG

TGGAGTAATGCTAATGAGTTGAGG

[n0033]

primer-circBBS9-R(SEQ ID NO.3)

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primer-circBBS9-R(SEQ ID NO.3)

[n0034]

GCTGAGACTTCAGGCATGG

GCTGAGACTTCAGGCATGG

[n0035]

Example 2: Expression level analysis of circBBS9 human homolog in osteoclasts

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实施例2circBBS9人类同源物在破骨细胞中的表达量分析

[n0036]

1. The human homolog of circBBS9 obtained in Example 1 was identified as has_circBBS9 by comparing it with the circBase database.

1、将实施例1得到的circBBS9通过circBase数据库比对得到人类同源物为为has_circBBS9。

The following specific primers were designed to detect the expression of has_circBBS9 in the lumbar spine of osteoporosis patients and osteoclasts differentiated from human peripheral blood cells by qPCR. It was found that has_circBBS9 was significantly upregulated, as shown in Figure 2.

设计如下特异性引物通过qPCR检测在骨质疏松患者腰椎和人外周血细胞分化而来的破骨细胞中的表达，发现has_circBBS9显著上调，见图2。

The sequence of has_circBBS9 is shown below (SEQ ID NO.4), and its PCR primers are shown in (SEQ ID NO.5) and (SEQ ID NO.6).

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has_circBBS9 的序列如下所(SEQ ID NO.4)示，其PCR引物如(SEQ ID NO.5)和(SEQ ID NO.6)所示。

hsa_circ_0134188(SEQ ID NO.4)

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hsa_circ_0134188(SEQ ID NO.4)

[n0037]

GTGGTTGTACTACAATCCCAGAGTCAGACCTAGAAGAAAGATCAGTAGAACAA

GACTCTACAGAACTGTTTACCAACCACAGACATCTCACTGCAGAGACACCCAGG

CCTGAAGTTTCACCCCTCCAAGGAGTCTCGGAATAATTCAAGTAGAGTTGTTTG

GTTGAGAGGAACATCCCCATCTCAAGGCCGAACCTGTGTGAACCTCATGCCAAG

CACAGATATAGGGCTGGCGCAGGTGCTTCCTAAAGCTCACCTTCCTGGAGATGA

CATGCATAGAAAGAGGGGTTGGGACTTTTTACTTCACTAGGAGAACTTGTAACA

CCATGGGGAAGTCAGCTGAAACTTGTCTTGTTTTGCCAGGAAAGGAAGTAGTTG

CCTTTGGTCATCCATCTGCTAATAGTCACAGAATACAGTGAAATGACATAGTTTT

GGGTTAGATTTTATAATGCAAAGATTCAGATCCAAAATAATTTCATACCCCATTT

TTTCACAGAATTCTTATATAGTAAATGTATCAAGTTTAATAAAGCATCTCATTGT

CAAATAATATCTTGGATTTTATTTATAATTAGAGGGATTTATGAGTGATTGCTCT

ACATTATTTCTTCAAAGGAAAGGAAAGGAATTGAAGACTTTGCTACTCTCTG

GTGGTTGTACTACAATCCCAGAGTCAGACCTAGAAGAAAGATCAGTAGAACAA

GACTCTACAGAACTGTTTACCAACCACAGACATCTCACTGCAGAGACACCCAGG

CCTGAAGTTTCACCCCTCCAAGGAGTCTCGGAATAATTCAAGTAGAGTTGTTTG

GTTGAGAGGAACATCCCCATCTCAAGGCCGAACCTGTGTGAACCTCATGCCAAG

CACAGATATAGGGCTGGCGCAGGTGCTTCCTAAAGCTCACCTTCCTGGAGATGA

CATGCATAGAAAGAGGGGTTGGGACTTTTTACTTCACTAGGAGAACTTGTAACA

CCATGGGGAAGTCAGCTGAAACTTGTCTTGTTTTGCCAGGAAAGGAAGTAGTTG

CCTTTGGTCATCCATCTGCTAATAGTCACAGAATACAGTGAAATGACATAGTTTT

GGGTTAGATTTTATAATGCAAAGATTCAGATCCAAAATAATTTCATACCCCATTT

TTTCACAGAATTCTTATATAGTAAATGTATCAAGTTTAATAAAGCATCTCATTGT

CAAATAATATCTTGGATTTTATTTATAATTAGAGGGATTTATGAGTGATTGCTCT

ACATTATTTCTTCAAAGGAAAGGAAAGGAATTGAAGACTTTGCTACTCTCTG

[n0038]

primer-has_circBBS9-F(SEQ ID NO.5)

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primer-has_circBBS9-F(SEQ ID NO.5)

[n0039]

AGAGGGATTTATGAGTGATTGCT

AGAGGGATTTATGAGTGATTGCT

[n0040]

primer-has_circBBS9-R(SEQ ID NO.6)

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primer-has_circBBS9-R(SEQ ID NO.6)

[n0041]

AGGTCTGACTCTGGGATTGT

AGGTCTGACTCTGGGATTGT

[n0042]

Example 3: Effects of siRNA^{circBBS9} transfection on different differentiation stages of osteoclasts

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实施例3转染siRNA^{circBBS9}后对破骨细胞不同分化阶段的影响

[n0043]

Design a circBBS9-specific siRNA, the sequence of which is shown below.

设计circBBS9特异性的siRNA，其序列如下所示。

After stimulating BMMs with RANKL for three days, siRNA^{circBBS9} was transfected into them, and RT-qPCR was used to analyze the marker genes of osteoclasts at different differentiation stages.

用RANKL刺激BMMs三天后，往其中转染siRNA^{circBBS9}，采用RT-qPCR对破骨细胞不同分化阶段的标记基因予以分析。

We found that after transfection with siRNA^{circBBS9}, the marker genes of osteoclasts in the multinucleation stage were significantly suppressed, while the marker genes of osteoclast precursor cells did not change significantly (see Figure 3).

我们发现转染siRNA^{circBBS9}后，破骨细胞的在多核化阶段的标记基因被显著抑制，而破骨细胞前体细胞的标记基因没有显著变化，见图3。

siRNA-circBBS9-sense(SEQ ID NO.7)：CUCUGGAGGUGGCUGUACUTT siRNA-circBBS9-antisense (SEQ ID NO.8)：AGUACAGCCACCUCCAGAGTT

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siRNA-circBBS9-sense(SEQ ID NO.7)：CUCUGGAGGUGGCUGUACUTT siRNA-circBBS9-antisense (SEQ ID NO.8)：AGUACAGCCACCUCCAGAGTT

[n0044]

Example 4: Effects of siRNA_NER17 transfection on osteoclast formation and function

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实施例4转染siRNA^{circBBS9}对破骨细胞形成和功能的影响

[n0045]

To further confirm the time period during which circBBS9 exerts its function, we transfected circBBS9 with siRNA^{circBBS9} on days 1, 3, and 5 after RANKL stimulation, and performed TRAP staining (PMID: 30378050) and bone resorption area measurement (PMID: 26592521).

为了进一步确认circBBS9究竟在哪个时间段发挥其功能，我们分别在RANKL刺激后第1、3、5天往其中转染siRNA^{circBBS9}，并予以TRAP染色(PMID:30378050)和骨吸收面积测定(PMID: 26592521)。

The results showed that transfection with siRNA^{circBBS9} on day 3 could maximally inhibit the formation and function of multinucleated osteoclasts, as shown in Figures 4 and 5.

结果表明第3天转染siRNA^{circBBS9}能够最大程度的抑制多核破骨细胞的形成和功能，结果如图4、5。

[n0046]

Example 5: Effects of siRNA^{circBBS9} transfection on osteoclast characterization gene expression

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实施例5转染siRNA^{circBBS9}对破骨细胞表征基因表达的影响

[n0047]

Western blot and qPCR were used to detect changes in the expression levels of osteoclast characterization genes transfected with siRNA^{circBBS9} on day 3, and the expression levels of osteoclast characterization genes were found to be significantly downregulated.

采用Western blot和qPCR检测第在3天转染siRNA^{circBBS9}的破骨细胞表征基因表达量的变化，发现破骨细胞表征基因的表达水平显著下调。

The results at the molecular level showed that osteoclast differentiation and function were significantly inhibited.

结果在分子水平上表明破骨细胞的分化和功能被显著抑制。

The results are shown in Figures 6 and 7.

结果如图6、图7所示。

[n0048]

Example 6: Effects of co-transfection of miR-423-3p mimic and siRNA^{circBBS9} on osteoclast multinucleation.

实施例6共转染miR-423-3p mimic和siRNA^{circBBS9}对破骨细胞多核化的影响

[n0049]

To further investigate the mechanism of action of circBBS9, miR-423-3p was identified as a downstream miRNA of circBBS9.

为进一步研究circBBS9的作用机制，miR-423-3p被拟定为circBBS9的下游miRNA。

siRNA^{circBBS9} significantly inhibited osteoclast multinucleation, however miR-423-3p sponge reversed this effect, suggesting that miR-423-3p is a downstream target of circBBS9.

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siRNA^{circBBS9}显著抑制了破骨细胞的多核化，然而miR-423-3p sponge逆转了这个效果，提示miR-423-3p是circBBS9的下游靶点。

The miR-423-3p sponge sequence is shown in (SEQ ID NO.9).

---

miR-423-3p sponge序列如(SEQ ID NO.9) 所示。

The results are shown in Figure 8.

结果图8所示。

[n0050]

siRNA-circBBS9-antisense(SEQ ID NO.9):

---

siRNA-circBBS9-antisense(SEQ ID NO.9)：

[n0051]

ACTGAGGGGCCTCAGACCGAGCT

ACTGAGGGGCCTCAGACCGAGCT

[n0052]

Example 7: circBBS9 can reduce bone loss in ovariectomized mice.

实施例7circBBS9能够减少去卵巢小鼠的骨质流失

[n0053]

To further clarify the functional effect of circBBS9 in osteoporosis, a biomimetic bone-targeting nanoparticle carrier (POCM-NPs@siRNA^{circBBS9}) was constructed.

为进一步明确circBBS9在骨质疏松中的功能效应，构建仿生骨靶向纳米颗粒载体(POCM-NPs@siRNA^{circBBS9})。

Sham and ovariectomized (OVX) osteoporosis models were established in mice and divided into three groups: sham group, OVX+siRNA^{NC} group (injected with POCM-NPs@siRNA^{NC} as a control), and OVX+siRNA^{circBBS9} group (injected with POCM-NPs@siRNA^{circBBS9}). The OVX was injected into mice one week after ovariectomy via tail vein. Femurs were harvested four weeks later for verification. The number and morphology of osteoclasts in mice were observed by TRAP staining and HE staining, and the histological condition of the tibia was observed by micro-CT.

建立sham以及小鼠去卵巢(OVX)骨质疏松模型，分为sham 组、OVX+siRNA^{NC}组(注射POCM-NPs@siRNA^{NC}作为对照)、OVX+siRNA^{circBBS9}组(注射 POCM-NPs@siRNA^{circBBS9})三组，通过尾静脉注射进入卵巢切除术后1周的小鼠体内，4周后取股骨验证，通过TRAP染色和HE染色观察小鼠破骨细胞数量和形态，通过micro-CT 观察小鼠胫骨骨组织学情况。

The results showed that, compared with POCM-NPs@siRNA^{NC}, knocking down circBBS9 reduced the number of osteoclasts and preserved the trabecular bone structure more completely (Figures 9 and 10), proving that knocking down this circRNA can alleviate osteoporosis.

结果显示，与POCM-NPs@siRNA^{NC}相比，敲减circBBS9可使破骨细胞数量减少，骨小梁结构保存更加完整(图9及图10)，证明敲减该circRNA 可减轻骨质疏松。

[n0054]

The above description is only a preferred embodiment of the present invention. It should be noted that for those skilled in the art, several improvements and modifications can be made without departing from the principle of the present invention, and these improvements and modifications should also be considered within the scope of protection of the present invention.

以上所述仅是本发明的优选实施方式，应当指出，对于本技术领域的普通技术人员来说，在不脱离本发明原理的前提下，还可以做出若干改进和润饰，这些改进和润饰也应视为本发明的保护范围。

[0060]

sequence list

序列表

[0061]

<110> Sir Run Run Shaw Hospital, Affiliated to Zhejiang University School of Medicine

---

<110> 浙江大学医学院附属邵逸夫医院

[0062]

<120> An osteoporosis biomarker circRNA and its application

<120> 一种骨质疏松标志物circRNA及其应用

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