Peptide-15 Changes miRNA Expression in Osteoblast-Like Cells

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Purpose: *Peptide-15 (P-15) is an analog of the cell-binding domain of collagen. P-15 has been shown to facilitate physiological process in a way similar to collagen, to serve as anchorage for cells, and to promote the binding, migration, and differentiation of cells. However, how P-15 alters osteoblast activity to promote bone formation is poorly understood. We therefore attempted to address this question by using microarray techniques to investigate the microRNA (miRNA) expression in osteoblasts exposed to P-15.*

Materials: *The miRNA oligonucleotide microarray provides a novel method to carry out genome-wide miRNA profiling in human samples. By using miRNA microarrays containing 329 probe designed from human miRNA sequence, we identified in osteoblast-like cells line (MG-63) cultured with P-15 several miRNA whose expression is significantly modified.*

Results: *We identified 11 up-regulated miRNA (*i.e.*, mir-337, mir-15b, mir-377, mir-100, mir-148a, mir-125a, mir-199a, mir-221, mir-let-7d, mir-92, mir-23b) and six down-regulated miRNA (*i.e.*, mir-422a, mir-19a, mir-224, mir-145, mir-22, mir-29a).*

Conclusion: *The data reported are, to our knowledge, the first on translation regulation in osteoblasts exposed to P-15. They can be relevant to better understand the molecular mechanism of bone regeneration and can serve as a model for comparing other materials with similar clinical effects. (Implant Dent 2008;17:100–108)*

Key Words: P-15, bone morphogenetic protein, miRNA, microarray, gene expression, gene profiling

Several research projects have been involved in the identification of factors that could help in the regeneration of lost tissue.[1] One avenue of research has been the identification of the specific cell-binding domain of type I collagen.[1] Type I collagen represents about one third of the body proteins.[2] Collagen, moreover, is a major determinant of the architecture and tensile strength of the tissues, and modulates cell proliferation, migration, differentiation, and specific gene expression.[2] Peptide-15 is an analog of the cell-binding domain of collagen.[2] P-15 competes for cell surface sites for attachment of collagen and, when immobilized on surfaces, it promotes adhesion of cells.[3] P-15 has been shown to facilitate physiological processes in a way similar to collagen, to facilitate the exchange of mechanical signals, and to promote cell differentiation.[4–6] Like other bone augmentation materials, P-15 associated with anorganic-derived bone matrix, has been shown to be helpful in the treatment of periodontal defects, and sinus-lifting procedures.[7–12]

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Because the mechanism by which P-15 stimulates osteoblast activity to promote bone formation is poorly understood, any information relative to bone biology could be helpful to reach a better comprehension of the clinical effect induced by P-15. From this point of view microRNAs (miRNAs) are a new field of research.

miRNAs represent a class of small, functional, noncoding RNAs of 19 to 23 nucleotides (nt) cleaved from 60 to 110 nt hairpin precursors.[13,14] Hundreds of miRNAs have been identified in plants and animals. The miRNAs are involved in various biological processes, including cell proliferation and cell death during development, stress resistance, and fat metabolism, through the regulation of gene expression[15] in posttranscriptional RNA silencing pathways. The RNA interference and the miRNA pathway regulate gene expression by inducing degradation or translational repression of target mRNAs. These pathways are generally initiated by various forms of double-stranded RNA, which are processed by Dicer, an RNase III family endonuclease, to 21 to 22 nt-long RNA molecules that serve as sequence-specific guides for silencing.[16,17]

miRNAs are transcribed as long primary transcripts, which are processed by a nuclear RNase III Drosha-containing complex into short hairpin

intermediates. These intermediate forms are transported to the cytoplasm where they are further processed by a second RNase III-family enzyme called Dicer to generate 22-bp RNA duplexes with 2-nt 3′ overhangs.[18–21]

miRNAs are loaded onto an Argonaute containing effectors ribonucleoprotein complex, referred to as miRNP or RNA-induced silencing complex, which is capable of recognizing cognate mRNAs and inhibiting protein expression.

Recent advances in spotted oligonucleotide microarray labeling and detection have enabled the use of this high-throughout technology for miRNA screening.

Microarray is a molecular technology that enables the analysis in parallel on a very large number of DNA or RNA fragments, spanning a significant fraction of the human genome. Gene expression is performed by a process of (i) miRNA extraction, (ii) labeling (different dyes are used for reference untreated cells and investigated cells—*i.e.*, cultured with P-15), and (iii) hybridization on slides containing miRNA probes. Then the slides are scanned with a laser system, and two false color images are generated for each hybridization with miRNA from the investigated and reference cells. The overall result is the generation of a so-called genetic portrait. It corresponds to up- or down-regulated miRNA in the investigated cell system.

We used a recently developed methodology for miRNA gene expression profiling based on the hybridization of a microchip, the Ncode Multi-Species miRNA Microarray, a slide printed with approximately 900 unique probe of miRNA sequences for *Homo sapiens*, *Mus musculus*, *Rattus norvegicus*, *Drosophila melanogaster*, *Caenorhabditis elegans*, and *Danio rerio*.

Because there is no study available concerning the effects of P-15 on RNA interfering, we compared miRNA expression (and consequently gene regulation) in human MG63 cells treated with P-15 versus untreated MG63 cells by the microarray analysis of the 329 human miRNAs sequences.

Materials and Methods

Cell Culture

Osteoblast-like cell (MG63) were cultured in sterile Falcon wells (Becton Dickinson, Franklin Lakes, NJ) containing Eagle's minimum essential medium supplemented with 10% fetal calf serum (Sigma Chemical Co., St. Louis, MO) and antibiotics (penicillin 100 U/mL and streptomycin 100 μg/mL; Sigma Chemical Co.). Cultures were maintained in a 5% CO_2 humidified atmosphere at 37°C.

MG63 cells were collected and seeded at a density of 1×10^5 cells/mL into 9 cm^2 (3 mL) wells by using 0.1% trypsin, 0.02% EDTA in Ca^{2+} and Mg-free Eagle's buffer for cell release. One set of wells were added with P-15 (Ceramed, Lakewood, CO) at the concentration of 10 μL/mL. P-15 was previously prepared by adding 2 mL serum-free medium in 1 g of P-15 for 1 hour at room temperature. After 24 hours, when cultures were subconfluent, cells were processed for RNA extraction.

miRNA Microarray

miRNA were extracted from the cells using the PureLink miRNA Isolation Kit (Invitrogen, Carlsbad, CA). From each sample (treated and control) 400 ng of miRNA was used for hybridization of NCode Multi-Species miRNA Microarray (Invitrogen), a slide containing 329 human miRNAs sequences in duplicate.

NCode miRNA Labeling System (Invitrogen) was used for labeling and hybridizing miRNA to microarray, according to the manufacturer's instructions. Briefly, a poly(A) tail was added to each miRNA, using a poly A polymerase and an optimized reaction buffer. Then, a capture sequence was ligated to the miRNA using a bridging oligo(dT). After a purification step, the tagged miRNAs were hybridized to the microarray and incubated overnight.

After an incubation of 18 to 20 hours, the array was washed and hybridized with Alexa Fluor 3 capture reagents (for the control) and Alexa Fluor 5 capture reagents (for the treated cells) and then switched. After another wash, the array was scanned using a standard microarray scanner (Axon Instruments, Sunnyvale, CA). After scanning, each spot is identified by means of GenePixR Array List file downloaded from www.invitrogen.com/ncode, which lists the identities and locations of all the probes printed on the array.

Images were quantified by GenePix 6.0 software (Axon Instruments). Signal intensities for each spot were calculated by subtracting local background from total intensities. The data were normalized by using the DNMAD and Preprocessing (http://gepas.bioinfo.cipf.es/cgi-bin/tools).[22,23] This generates an average value of the two spot replicates of each miRNA.

To select the differentially expressed miRNA, the data obtained were analyzed using the Significance Analysis of Microarray package.[24]

For target predictions and validations, miRNA were processed using miRBase Target, a Web resource (http://microrna.sanger.ac.uk/targets/v4/) developed by the Enright Lab at the Wellcome Trust Sanger Institute. This source uses an algorithm called miRanda to identify potential binding sites for a given miRNA in genomic sequences.

The gene target list was then processed by FatiGO (http://fatigo.bioinfo.cnio.es), a Web interface that carries out simple data mining using gene ontology. The data mining consists on the assignation of the most characteristic gene ontology term to each cluster of regulated genes.

Results

Hybridization of miRNA (derived form MG63 cultured with P-15 at the concentration of 10 μL/mL) to the sequences spotted on the slide allowed us to perform systemic analysis of miRNAs and to provide primary information with regard to the regulation of translation process induced by P-15. There were 11 up-regulated miRNA (*i.e.*, mir-337, mir-15b, mir-377, mir-100, mir-148a, mir-125a, mir-199a, mir-221, mir-let-7d, mir-92, mir-23b) and six down-regulated miRNA (*i.e.*, mir-422a, mir-19a, mir-224, mir-145, mir-22, mir-29a) for false discovery rate = 0 and score >14. Figure 1 is the graphic output of Significance Analysis for Microarray and shows differentially expressed miRNA. Because miRNA potentially regulates thousands of genes, in this study, we selected only genes related to osteogenesis and bone remodeling (Table 1). Genes with opposed regulation were excluded.

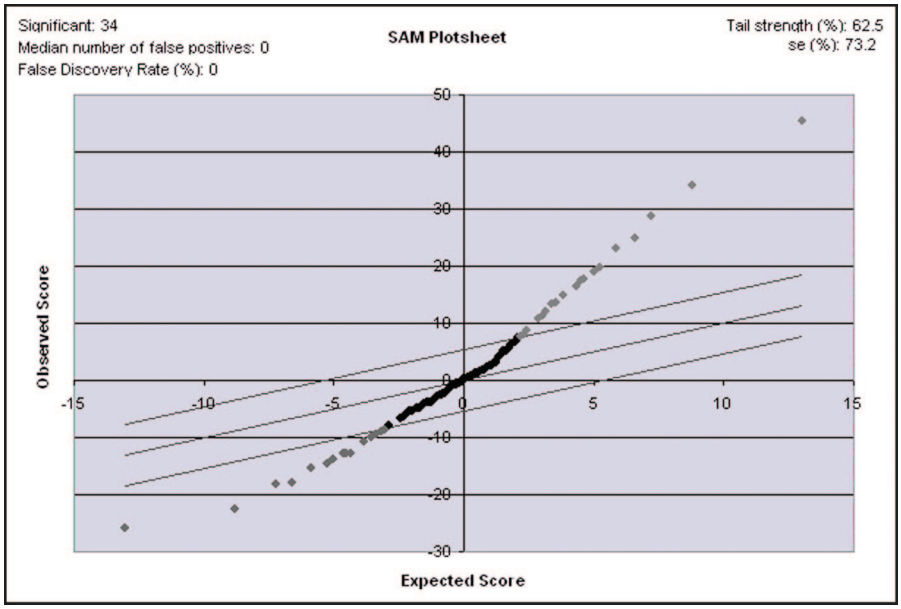

Fig. 1. Significance Analysis of Microarray plot of MG63 cultured for 24 hours with P-15 at the concentration of 10 μL/mL. Expected differentially expressed miRNAs are reported in *x* axis whereas observed differentially expressed miRNAs are in *y* axis. Down-regulated miRNAs are located in the lower left side of the diagram; up-regulated miRNAs are in the upper right side; miRNAs with different expression but statistically not significant are black dots. Parallel lines drowned from lower-left to upper-right squares are the cut-off limits. The solid line indicates the equal value of observed and expected differentially expressed miRNAs.

Table 1. Down- and Up-Regulated Genes in MG63 Cultured for 24 Hours With P-15 at the Concentration of 10 μL/mL

		Target Genes	
miRNA	Biological Function	Symbol	Name
Up-regulated	Skeletal development	HOXD13	Homeobox D13
		AEBP1	AE binding protein 1
		SHOX	Short stature homeobox
		EN1	Engrailed homolog 1
		COMP	Cartilage oligomeric matrix protein
		SUFU	Suppressor of fused homolog
		IGF1	Insulin-like growth factor 1
		MATN1	Matrilin 1, cartilage matrix protein
	Cartilage development	NOG	Nogging
Down-regulated	Bone formation	TFIP11	Tuftelin interacting protein 11

Discussion

P-15 is an highly conserved linear peptide with a 15-amino acid sequence identical to the sequence contained in the residues 766 to 780 of the α-chain of type I collagen.[1] To get more information concerning the manner in which P-15 alters osteoblast activity to promote bone formation, we attempted to address this question by using a new method, miRNA microarray.

miRNAs are a recently discovered class of small, ~19 to 23-nucleotide noncoding RNA molecules. They are cleaved from 70 to 110-nucleotide hairpin precursors and play an important role in the posttranscriptional regulatory process. miRNAs are not translated into proteins; instead, they regulate the expression of other genes by either cleaving or repressing the translation of their mRNA targets.

Hybridization of miRNA derived from MG63 cultured with 10 μL/mL of P-15 to the sequences spotted on the slide allowed us to perform systemic analysis of miRNAs and to provide primary information concerning regulation of translation induced by P-15 (Table 1).

Most of detected genes are down-regulated and among them are some homeobox genes (*i.e.*, genes that regulate the morphogenesis of part of the body). Noggin or NOG inactivates members of the TGF-β superfamily signaling proteins, such as BMP4. By diffusing through extracellular matrices more efficiently than members of the TGF-β superfamily, noggin may have a principal role in creating morphogenic gradients.[25] HOXD13 belongs to the homeobox family of genes named HOX and is divided in four groups (from A to D). Mutations in HOXD13 cause synpolydactyly.[26] SHOX (Short stature Homeobox-containing gene) is involved in idiopathic growth retardation and in the short-stature phenotype of Turner syndrome patients.[27] The human engrailed homologs 1 (*i.e.*, EN1) is implicated in the control of pattern formation during limb development.[28] Another group of down-regulated genes are hormones or cytokines. IGF1 (or insulin-like growth factors) mediates many of the growth-promoting effects of growth hormone.[29] GDF11 is a member of the BMP family and the transforming growth factor (TGF)-β superfamily. It has a role in mesodermal formation and neurogenesis during embryonic development.[30] Finally, COMP is a noncollagenous extracellular matrix protein and its mutations cause the osteochondrodysplasias pseudoachondroplasia and multiple epiphyseal dysplasia.[31]

Conclusion

The genes discussed are only a limited number among those differentially regulated by miRNA reported in Table 1. We briefly described some of those with a better-known function and directly related to bone formation, skeletal development, cartilage remodeling, and bone production. In addition, the fact that several genes related to bone formation have a translational negative control can be related to the early stage analyzed (*i.e.*, MG63 cultured for 24 hours until they are subconfluent). This phase is characterized by elevated rates of mitosis and a low extracellular matrix production and differentiation.

It is worth noting that MG63 are a cell line and not normal osteoblasts. Notwithstanding the advantages of us-

ing a cell line is related to the fact that the reproducibility of the data is higher as compared with the same number of primary cultures. In fact in this case, as there is a variability related to the patient it will be necessary to have a higher number of cultures (*i.e.*, samples from patients). Primary cell cultures provide a source of normal cells but they also contain contaminating cells of different types and cells in variable differentiation states. Moreover, we have chosen to perform the experiment after 24 hours to get information on the early stages of stimulation. It is our understanding, therefore, that more investigations with other osteoblast-like cell lines, primary cultures, and different time points are needed to get a global comprehension of the molecular events related to P-15 action. The reported data can be a model to compare different substances with similar effects.

Disclosure

The authors claim to have no financial interest in any company or any of the products mentioned in this article.

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ID Abstract Translations

GERMAN / DEUTSCH

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Peptide-15 verändert den Ausdruck von miRNA in osteoblastartigen Zellen

ZUSAMMENFASSUNG: Zielsetzung: Peptide-15 (P-15) stellt einen Analogstoff der zellbindenden Domäne des Kollagen dar. P-15 hat dabei Eigenschaften hinsichtlich der Erleichterung des physiologischen Prozesses vergleichbar dem Kollagen bewiesen und dient damit als Anker für Zellen und fördert die Anbindung, Migration sowie Differenzierung der Zellen. Allerdings besteht nach wie vor wenig Verständnis dafür, auf welche Weise P-15 die Osteoblastenaktivität verändert, um darüber eine Förderung der Knochengewebsbildung zu erzielen. Daher haben wir versucht uns dieser Frage zuzuwenden. Über Mikrogruppierungstechniken wurde der microRNA-Ausdruck in dem Stoff P-15 ausgesetzten Osteoblastenzellen untersucht. ***Materialien und Methoden:*** Die Mikrogruppierung des miRNA-Oligonukleotids bietet eine neuartige Methode zur genomweiten Erstellung von microRNA-Profilen am Menschen. Durch die Verwendung von miRNA-Mikrogruppierungen mit 329 Proben, die aus menschlichen miRNA-Sequenzen gewonnen wurden, fanden wir verschiedene miRNA mit maßgeblich veränderten Ausdrücken in der mit P-15 kultivierten Osteoblastenartigen Zelllinie (MG-63). ***Ergebnisse:*** Wir bestimmten 11 nach oben hin regulierte miRNA (d. h. mir-337, mir-15b, mir-377, mir-100, mir-148a, mir-125a, mir-199a, mir-221, mir-let-7d, mir-92, mir-23b) sowie 6 nach unten regulierte miRNA (i.e. mir-422a, mir-19a, mir-224, mir-145, mir-22, mir-29a). ***Schlussfolgerung:*** Die in diesem Bericht zusammengetragenen Daten machen unseres Wissens nach die erste Studie hinsichtlich der Translationsregulierung bei dem Stoff P-15 ausgesetzten Osteoblastenzellen aus. Sie können wesentlich zu einem besseren Verständnis der molekularen Abläufe der Knochengewebsregeneration beitragen sowie als Modell zum Vergleich anderer Materialien mit ähnlichen klinischen Effekten dienen.

SCHLÜSSELWÖRTER: P-15; morphogenetisches Knochengewebsprotein; miRNA; Mikrogruppierung; Genausdruck; genetisches Profil

SPANISH / ESPAÑOL

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El Péptido-15 cambia la expresión de miARN en células similares a osteoblastos

ABSTRACTO: Propósito: El Péptido-15 (P-15) es un compuesto análogo del grupo de aglutinadores de células del colágeno. El P-15 ha demostrado facilitar el proceso fisiológico de manera similar al colágeno, para servir como anclaje a las células y promover el aglutinamiento, migración y diferenciación de las células. Sin embargo, no se entiende con claridad cómo altera el P-15 la actividad del osteoblasto para promover la formación de hueso. Por lo tanto, intentamos ocuparnos de esta pregunta usando una técnica de micromatriz para investigar la expresión microARN en los osteoblastos expuestos a P-15. ***Materiales y Métodos:*** La micromatriz oligonucleótida de miARN proporciona un novedoso método para completar un perfil de microARN en todo el gen en muestras humanas. Al usar micromatrices de miARN que contienen 329 sondas diseñado de la secuencia de microARN humano, identificamos en la línea de células similares a osteoblastos (MG-63) cultivadas con P-15 varios miARN cuya expresión está significativamente modificada. ***Resultados:*** Identificamos 11 miARN regulados hacia arriba (o sea, mir-337, mir-15b, mir-377, mir-100, mir-148a, mir-125a, mir-199a, mir-221, mir-let-7d, mir-92, mir-23b) y 6 miARN regulados hacia abajo (o sea, mir-422a, mir-19a, mir-224, mir-145, mir-22, mir-29a). ***Conclusión:*** Los datos incluidos son, según nuestros conocimientos, el primer estudio sobre la regulación de la traducción en osteoblastos expuestos a P-15. Pueden ser relevantes para entender mejor el mecanismo molecular de la regeneración de hueso y como modelo para comparar otros materiales con efectos clínicos similares.

PALABRAS CLAVES: Péptido P-15; proteína morfogenética del hueso; miARN; micromatriz; expresión del gen; perfil del gen

PORTUGUESE / PORTUGUÊS

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Peptídeo-15 altera a expressão de miRNA em células to tipo osteoblasto

RESUMO: Objetivo: O Peptídeo-15 (P-15) é um análogo do domínio de colágeno de ligação da célula. O P-15 mostrou facilitar o processo fisiológico de modo semelhante ao colágeno, servir como ancoragem para células e promover a ligação, migração e diferenciação de células. Contudo, como o P-15 altera a atividade do osteoblasto para promover a formação de osso é deficientemente compreendido. Portanto, tentamos tratar essa questão usando técnicas de microarranjo para investigar a micro expressão de RNA em osteoblastos expostos a P-15. ***Materiais e Métodos:*** O microarranjo do oligonucleotídeo de miRNA fornece um método moderno de realizar perfilamento de microRNA com abrangência do genoma em amostras humanas. Usando microarranjos de miRNA contendo 329 sondas projetadas a partir de seqüência de miRNA Humano, identificamos na linha de células do tipo osteoblasto (MG-63) cultivada com P-15 vários miRNA cuja expressão está significativamente modificada. ***Resultados:*** Identificamos 11 miRNA regulados para cima (i.e. mir-337, mir-15b, mir-377, mir-100, mir-148a, mir-125a, mir-199a, mir-221, mir-let-7d, mir-92, mir-23b) e 6 miRNA regulados para baixo (i.e. mir-422a, mir-19a, mir-224, mir-145, mir-22, mir-29a). ***Conclusão:*** Os dados relatados são, até onde conhecemos, o primeiro estudo sobre regulação da tradução em osteoblastos expostos a P-15. Eles podem ser relevantes para melhor entender o mecanismo molecular da regeneração do osso e como modelo para comparar outros materiais com efeitos clínicos semelhantes.

PALAVRAS-CHAVE: P-15; proteína morfogenética do osso; miRNA; microarranjo; expressão do gene; perfilamento do gene

RUSSIAN / РУССКИЙ

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Воздействие Пептида-15 на экспрессию микроРНК в остеобластоподобных клетках

РЕЗЮМЕ: **Цель.** Пептид-15 (П-15) является аналогом клеточносвязывающего домена коллагена. Установлено, что П-15, аналогично коллагену, способствует протеканию физиологических процессов, выступает в качестве опоры для клеток и стимулирует их связывание, перемещение и дифференциацию. Однако до сих пор не вполне понятно, каким образом П-15 изменяет активность клеток костной ткани, тем самым способствуя формированию костей. Поэтому мы попытались найти ответ на этот вопрос, используя методику биочипа, чтобы рассмотреть экспрессию микроРНК клеток костной ткани, которые подвергались воздействию П-15. **Материалы и методы.** Биочип на основе олигонуклеотидов микроРНК позволяет создать оригинальную методику для определения геномного профиля микроРНК человека. Используя биочипы микроРНК, которые содержали 329 контактов, созданных на основе последовательности человеческой микроРНК, мы выявили в линии остеобластоподобных клеток, искусственно выращенных с применением П-15, несколько микроРНК, экспрессия которых была значительно изменена. **Результаты.** Мы выявили 11 активированных микроРНК (например: mir-337, mir-15b, mir-377, mir-100, mir-148a, mir-125a, mir-199a, mir-221, mir-let-7d, mir-92, mir-23b) и 6 подавленных микроРНК (например: mir-422a, mir-19a, mir-224, mir-145, mir-22, mir-29a). **Вывод.** Насколько нам известно, данные нашего исследования являются первыми данными по регулированию трансляции в клетках костной ткани, подвергнутых воздействию П-15. Эти данные важны для лучшего понимания молекулярного механизма регенерации кости и могут послужить в качестве сравнительной модели для других материалов со схожим клиническим эффектом.

КЛЮЧЕВЫЕ СЛОВА. П-15, костный морфогенетический белок, микроРНК, биочип, экспрессия гена, профиль гена

TURKISH / TÜRKÇE

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Peptid-15, osteoblast benzeri hücrelerde miRNA ekspresyonunu deðiþtirir

ÖZET: **Amaç:** Peptid-15 (P-15), kollajenin hücreye bağlanan alanının bir analogudur. P-15′in kollajene benzer şekilde fizyolojik süreci kolaylaştırdığı, hücreler için bir ankraj rolü oynadığı ve hücrelerin bağlanmasını, migrasyonunu ve ayırımını ilerlettiği gösterilmiştir. Ancak, P-15′in osteoblast ak-

tivitesini nasıl değiştirerek kemik formasyonunu ilerlettiği halen iyi bir şekilde anlaşılamamaktadır. Bu nedenle amacımız, mikrotest (microarray) tekniklerini kullanarak P-15′e maruz kalmış osteoblastlarda mikroRNA ekspresyonunu araştırmaktı. **Gereç ve Yöntem:** miRNA oligonükleotid mikrotesti, insan örneklerinde genom-çapında mikroRNA profillemesi için yeni bir yöntem sunar. İnsan miRNA sekansından tasarlanan 329 prob içeren miRNA mikrotestlerini kullanarak, P-15 ile ekim (kültür) yapılmış osteoblast benzeri hücre (MG-63) hattında ekpresyonu önemli ölçüde değişmiş birçok miRNA tanımladık. **Bulgular:** 11 up-regülasyonlu miRNA (örn. mir-337, mir-15b, mir-377, mir-100, mir-148a, mir-125a, mir-199a, mir-221, mir-let-7d, mir-92, mir-23b) ve 6 down-regülasyonlu miRNA (örn. mir-422a, mir-19a, mir-224, mir-145, mir-22, mir-29a) tanımladık. **Sonuç:** Bilgimize göre burada bildirilen veriler, P-15′e maruz kalmış osteoblastlarda çeviri regülasyonunun ilk çalışmasını oluşturmaktadır. Bu veriler, kemik rejenerasyonunun moleküler mekanizmasını anlamaya yardımcı olabileceği gibi, benzer klinik etkileri olan diğer materyallerle karşılaştırmalarda model de olabilir.

ANAHTAR KELÝMELER: P-15; kemik morfogenetik protein; miRNA; mikrotest; gen ekspresyonu; gen profilleme

JAPANESE / 日本語

ペプチド-15がosteoblast-like細胞内在miRNAの発現を変化

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研究概要：

目的: ペプチド-15(P-15)15アミノ酸残基)はコラーゲンの細胞接着分子類似体である. P-15はコラーゲンと類似したかたちで生理プロセスを助成し、細胞を固定、さらに細胞の接着、移動、分化を促進することが明らかになっている。しかしながら P-15がどのような方法で骨芽細胞活動を変質し骨形成を促進するかについてはまだ充分な理解が得られていない。そこで我々はこの疑問に取組むためにマイクロアレイ技術を利用して P-15の影響を受けた骨芽細胞に内在するmicroRNAの発現調査を行った。

研究素材と方法: miRNA合成オリゴヌクレチド　マイクロアレイはヒトサンプルのゲノムワイド　microRNAプロファイリングに斬新な方法を提供する。ヒトのmiRNAシーケンスをもとにデザインされた329のプローブを含むmiRNAマイクロアレイを使用し、P-15と培養したosteoblast-like細胞ライン (MG-63)に著しい発現変化が確認できるいくつかのmiRNAを識別した。

結果: 研究で識別されたのは次の通りである：11 up-regulated miRNA (i.e. mir-337, mir-15b, mir-377, mir-100, mir-148a, mir-125a, mir-199a, mir-221, mir-let-7d, mir-92, mir-23b) 、これに加え 6 down-regulated miRNA (i.e. mir-422a, mir-19a, mir-224, mir-145, mir-22, mir-29a).

結論:　我々の認識する限りでは、ここで報告する当データが　P-15に影響を受けた骨芽細胞翻訳制御に関する初の研究データである。当データは骨組織再形成の分子メカニズムにいっそうの理解を深めると共に、類似臨床効果を備える他の素材比較基準としても適応できる。

キーワード：　P-15; 骨組織形態形成タンパク質;　miRNA; マイクロアレイ;　遺伝子発現;　遺伝子プロファイリング

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CHINESE / 中国語

Peptide-15 蛋白質改變類造骨細胞中的微型核糖核酸表現

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摘要：

目的：Peptide-15 (P-15) 蛋白質是一種膠原蛋白細胞結合區域的類似物。P-15 已經顯示能促進與膠原蛋白類似的生理作用，可做爲細胞的連接物並促進細胞結合、轉移和差異化。然而，有關 P-15 如何改變造骨活動來促進骨成形，我們所知有限。因此，爲了回答此問題，我們嘗試利用微陣方法來研究暴露於 P-15 的造骨細胞中的微型核糖核酸的表現。

資料與方法：微型核糖核酸寡核酸微陣提供一套新方法，以在人體樣本進行基因體微型核糖核酸分析。

藉著使用包含從人體微型核糖核酸排序設計的 329 探針的微型核糖核酸微陣，我們找出以蛋白質 P-15 培育的類造骨細胞列 (MG-63) 中的多種表現已顯著修改的微型核糖核酸。

結果：我們找到 11 種上調的微型核糖核酸（即 mir-337、mir-15b、mir-377、mir-100、mir-148a、mir-125a、mir-199a、mir-221、mir-let-7d、mir-92、mir-23b）和 6 種下調的微型（即 mir-422a、mir-19a、mir-224、mir-145、mir-22、mir-29a）。

結論：據我們所知，此報告的數據是第一個有關暴露於 P-15 蛋白質的造骨細胞的翻譯調控，可能與更加了解骨質再生的分子機轉有關，也可做爲比較其他資料與類似臨床作用的模型。

關鍵字：P-15、**骨成形蛋白質、微型核糖核酸、微陣、基因表現、基因分析**

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KOREAN / 한국어

유사 조골세포 내miRNA발현을 변화 시키는 펩타이드-15(Peptide-15)

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초록

목적: 펩타이드-15(P-15)는 콜라겐의 세포를 결합하는 영역의 유사체이다. P-15는 세포에 고착되어 세포의 결합과 이동, 분화를 진행시킴으로써 콜라겐과 유사한 방식으로, 생리학적 과정을 촉진시키는 것으로 나타났다. 그러나 골 형성 촉진을 위해 P-15이 어떻게 조골세포 활동을 변경시키는지는 분명히 이해가 되지 않는다. 따라서 P-15에 노출된 조골세포의 microRNA 연구를 위해 미세배열법을 이용하여 본 문제를 해결하고자 했다.

재료 및 방법: miRNA 올리고핵산염 미세배열은 인간을 표본으로 유전체 microRNA 프로파일링을 수행하는 새로운 방법을 제공한다.

인간 miRNA 순서에서 개발된 329 탐침이 포함된 miRNA 미세배열을 이용하여, 발현이 유의하게 수정된 여러 P-15 miRNA와 배양된 유사 조골세포주 (MG-63)을 확인하였다.

결과: 11개의 상향 조절된 miRNA (즉, mir-337, mir-15b, mir-377, mir-100, mir-148a, mir-125a, mir-199a, mir-221, mir-let-7d, mir-92, mir-23b) 및 6개의 하향 조절된 miRNA (즉, mir-422a, mir-19a, mir-224, mir-145, mir-22, mir-29a)를 확인하였다.

결론: 보고된 자료는 분명 P-15에 노출된 조골세포의 해독 조절에 대한 첫 연구이다. 골 재생의 분자 기전에 대한 이해와 관련이 있을 수 있으며, 유사한 임상 효과와 기타 자료를 비교하기 위해 모델이 될 수 있다.

핵심 단어: P-15; 골 형태형성 단백질; miRNA; 미세배열; 유전자 발현; 유전자 프로파일링

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