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Chapter 5

Vitamin K2: A Vitamin that Works like a Hormone, Impinging on Gene Expression

Jan Oxholm Gordeladze

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.80388

Abstract

Vitamin K2 binds to the intranuclear receptor SXR and results in the activation of a plethora of genes, both directly and indirectly. Among these genes are important biological markers of cellular characteristics or features (also known as cell phenotypes), as well as a set of molecules known to be involved in both hormone-induced, G-protein-mediated cell signalling, either directly or indirectly activating so-called sirtuins and/or histone deacetylaces (HDACs), known as determinants of cell types and their specific functions in a given tissue. Hence, vitamin K2 may be closely involved in or serving as a traditional molecular 'link' between hormonal receptors and intracellular signalling pathways. It has been stated that a true hormone is a product of living cells, which circulates in body fluids (such as blood) and elicits a specific and often stimulatory effect on the activity of cells situated remotely from its point of origin. A large bulk of evidence published over the past 10 years establishes vitamin K2 in this category of substances. Hence, vitamin K2 should be considered and consequently classified as a hormone.

Keywords: vitamin K2, SXR, G-proteins, vitamin A-D-K2 cascade, bioinformatics, in vitro model systems

1. Introduction

Vitamin K has since more than 25 years been known to serve as a powerful nutrient factor in the preservation of homeostatic bone turnover, along with blood clotting biochemistry. In addition, vitamin K has been used as a therapeutic remedy in the clinic to treat and prevent bone brittleness (osteoporosis) in Japan and many other countries around the world. Moreover, beyond its enzymatic character as a cofactor for the vitamin K-dependent GGCX (gamma-glutamyl carboxylase), Professor Inoue and his co-workers have shown that the K2 variant is,

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in fact, a transcriptional modulator of osseous marker genes, also serving as an extracellular matrix-related molecule, via the stimulation of the 'steroid and xenobiotic receptor' SXR [1]. A microarray-based revelation of the present action of vitamin K2 in bone-derived osteoblastic cell genes corroborated the notion that the K2 variant menaquinone-4 (MK-4), in fact, is a hormone. Among the significantly upregulated genes, both growth differentiation factor 15 (GDF15) and stanniocalcin 2 (STC2) were concluded to serve as novel target genes, which both circumvented the traditional GGCX- and SXR-mediated pathways found in osteoblast-like cells.

2. Vitamin K2: vitamin and hormone

The induction of both GDF15 and STC2 genes is construed as specific to MK-4, since it was shown not to be brought about by another vitamin K(2) isoform MK-7, vitamin K(1) or the MK-4 side chain containing the geranylgeraniol group. A survey into the signalling pathways in question indicated that MK-4 sustained phosphorylation of protein kinase A (PKA) and that MK-4 mediated upregulation of genes, such as GDF15 and STC2, was diminished by the exposure to a PKA inhibitor or by siRNA constructs against PKA. These observations were in line with the concept that vitamin K(2) was capable of modulating its own target gene expression in bone-derived (osteoblastic) cell entities through a PKA-driven pathway, which in essence was different from the traditional vitamin K-dependent signalling pathways [2].

Vitamin K2 has been included as a member in a group of molecules, constituting the 'requirement' for blood to coagulate; however, it has been demonstrated to function or serve as a key in the homeostasis of osseous tissue, thus showing effectiveness as one therapeutic agent, among others, in the curative treatment portfolio of bone ailments and diseases, i.e. like bone loss or brittleness (osteomalacia and osteoporosis). Furthermore, it has, since several decades, been acknowledged that vitamin K2 mediates transcriptional modulation of marker genes in osteoblastic cells, as well as reinforcing bone formation via the nuclear steroid and xenobiotic receptor, SXR. In this context, Dr. Satoshi Inoue and his research team identified several genes, which were upregulated by vitamin K2 (and a prototypical SXR like ligand, rifampicin) in osteoblastic cells, through microarray analysis and PCR. A plethora of genes was upregulated, among which collagen synthesis and accumulation in osteoblast-like MG63 cells were enhanced several times over by vitamin K2 treatment. Therefore, the results of Dr. Inoue and his research group more than suggested a novel function for vitamin K2 in the formation of osseous tissues, i.e. that K2 was a true transcriptional regulator of extracellular matrix-related genes, being involved in the assembly of collagen. At present, we know that vitamin K2 (or menaquinone-7 = MK-7, among other vitamin K2 metabolites) works through this nuclear receptor and consequently should be classified as a hormone and not solely be construed as 'a vitamin'.

Arterial stiffness is always associated with an enhanced cardiovascular risk, morbidity and mortality [3]. The present article reviews the main vitamins being involved in arterial stiffness and enabling destiffening; their mechanism of action, providing a brief description of the latest studies in the area; and their implications for primary cardiovascular prevention, clinical practice and therapy. Despite inconsistent proof for 'softening' brought about by vitamin supplementation in a plethora of clinical trials, promising results were observed in

selected populations. The chief mechanisms pertain to anti-atherogenic potential, substantial augmentation of endothelial functionality (pertaining to vitamins A, C, D and E, respectively) and general metabolic profiling (pertaining to vitamins A, B12, C, D and K, respectively), suppression of the renin-angiotensin-aldosterone (R-R-A) system (vitamin D), anti-inflammatory (vitamins A-D-E-K) and antioxidant effects (vitamins A-C-E), diminished homocysteine levels (vitamin B12) and a reversal of the calcification of arteries (vitamin K). Vitamins A, B12, C, D and E, as well as vitamin K status, are important in evaluating the risk of cardiovascular events, and, finally, supplementation with vitamins may serve as an efficient, individually based and less costly 'destiffening' therapeutic mode.

Vitamin K is renowned for being an important (vital) nutrient, sustaining both bone homeostasis and blood coagulation. Therefore, it is both clinically and ubiquitously used as a therapeutic agent or treatment for osteoporosis in Japan and western countries. Besides its powerful enzymatic, cofactor role of the vitamin K-dependent g-glutamyl carboxylase (GGCX), it has since long been known that vitamin K2 may serve as a transcriptional regulator of marker osteoblastic genes, as well as in matrix-related, extracellular genes. In this context the activation of the so-called steroid and xenobiotic receptor (known as SXR) is mandatory.

Hence, genes known to be upregulated by vitamin K2 isoforms like menaquinone-4 (MK-4) were applied using oligonucleotide-based microarray analyses. Among the MK-4 upregulated gene species, the growth differentiation factor 15 (GDF15) and stanniocalcin 2 (STC2) were discovered as new MK-4 target genes, being independent of the GGCX and SXR pathways in osteoblastic cells. The observed induction of GDF15 and STC2 was construed as specific to MK-4, since it was not seen with exposure to MK-7 or vitamin K1. Surprisingly, a scrutiny of the main signalling pathways showed that MK-4 stimulated PKA (protein kinase A) phosphorylation. Furthermore, the MK-4-dependent induction of both GDF15 and STC2 genes was obliterated subsequent to the treatment with PKA inhibitors or siRNAs against PKA. Therefore, it was postulated that vitamin K2 (MK-7) could modulate target gene expression in osteoblastic cells via PKA-dependent mechanisms, which were distinct from any previously known vitamin K-mediated signalling pathway.

The paper found that vitamin K2 is recognised, along with calcium, vitamin D and magnesium, as essential in supporting strong bones and healthy arteries. In the paper, *Nutritional strategies for skeletal and cardiovascular health: hard bones, soft arteries, rather than vice versa*, the authors cite a US Surgeon General's Report that states that one in two Americans over 50 is expected to have or to be at risk of developing osteoporosis, which causes 8.9 million fractures annually, with an estimated cumulative cost of incident fractures predicted at $474 billion during the next 20 years in the USA.

Furthermore, a study conducted by the Mayo Clinic [4, 5] reported that 'compared with 30 years ago, forearm fractures have risen more than 32% in boys and 56% in girls'. Meanwhile, strong epidemiological associations exist between decreased bone mineral density (BMD) and increased risk of cardiovascular (CV) disease. For example, individuals with osteoporosis have a higher risk of coronary artery disease and vice versa. This problem will be magnified, according to the paper, if the therapies for osteoporosis (calcium supplements) independently increase risk of myocardial infarction. To that end, the authors conducted a comprehensive and

systematic review of the scientific literature to determine the optimal dietary strategies and nutritional supplements for long-term skeletal health and cardiovascular health. They summarised what is helpful for building strong bones while maintaining soft and supple arteries:

- Obtain calcium from dietary sources (the best choice is a calcium hydroxyapatite) with the adequate animal protein, fruit and vegetable intake.
- Concomitantly increase potassium consumption, while reducing sodium intake should be taken into account.
- Maintain vitamin D levels in the normal range.
- Increase the intake of foods rich in vitamins K1 and K2.

The study notes: 'A meta-analysis concluded that while supplementation with phytonadione (vitamin K1) improved bone health, vitamin K2 was even more effective in this regard. This large and statistically rigorous meta-analysis concluded that high vitamin K2 levels were associated with reduced vertebral fractures by approximately 60%, hip fractures by 77% and all non-vertebral fractures by approximately 81%. Supplementation with vitamin K2 as MK-7 increased bone strength in postmenopausal women in 3-year clinical study'.

'Additionally, increased vitamin K2 intake has been associated with decreased arterial calcium deposition and the ability to reverse vascular calcification in animal models. Moreover clinical trial proved that vitamin K2 supplementation increases elasticity of the arteries (in 3 years)', the paper stated. The authors recommend increasing the intake of foods rich in vitamins K1 and K2 to secure skeletal and cardiovascular health. 'The positive health potential of vitamin K2 is more effective than for vitamin K1, the paper reads. Yet, Dr. Hogne Vik, Chief Medical Officer with NattoPharma, world leader in vitamin K2 R&D, exclusive global supplier of MenaQ7 vitamin K2 as MK-7, and sponsor of the 3-year studies cited in the paper, explains that it is not possible to get sufficient amounts of vitamin K2 through a European or US diet'.

The only food that contains enough vitamin K2 is the Japanese dish Natto. 'This means that if you want to get enough vitamin K2 into your body, then you have to take dietary supplements or functional foods containing vitamin K2', he said. 'We are gratified, but not surprised, that our 3-year clinical studies were cited in this paper', Dr. Vik continues. 'NattoPharma has driven the clinical research that has demonstrated vitamin K2's benefits for human health, and our breakthrough studies provided the first intervention data confirming the associations that observational studies made previously: that vitamin K2 as MK-7 is available beyond the liver to support bone and cardiovascular health. And it does this by activating proteins that help the body to properly utilise calcium – there by simultaneously supporting both skeletal and cardiovascular health'.

How are we going to interpret the above-described information about the mechanism of action of vitamin K2? Well, according to the definition given in dictionaries and scientific papers, vitamin K2 (as in the form or MK-4 or MK-7) fits this definition and should be classified as one.

3. What features characterise a true hormone?

To make the picture clearer, let us start all over again. According to **Figure 1**, the impact of a hormone is one signal among differentiation signals (also called epigenator factors), which may be temperature variations, oxygen tension, mechano-stimulation or humoral factor/hormones. The 'hormone' eventually activates a transcription factor or microRNA synthesis, which may impinge upon DNA in terms of a certain or given spectrum of mRNAs appearing in the cytosol of the cell. Of major interest here are eventually two classes of molecules utmost important for the acquisition of the final cell phenotype—the histones and the sirtuins (see **Figure 5**).

The response elicited by a single hormone (epigenator) may look like the network of interacting factors (mostly transcription factors), such as the network representing the closely cooperating network (mostly represented by transcription factor), as seen in **Figure 2a** and **b**.

The reader is recommended to look up the remainder of the genes shown above and will be amazed as to the plethora of biological effects being modulated (directly and/or indirectly) by the nuclear receptor NR1/NR2 = SXR, to which vitamin K2 binds, exerting its multitude of biological effects. The crucial question is then: what may be the immediate effect of vitamin K2 (e.g. MK-7) on cell phenotype, for example, on bone chips harbouring live osteoblastic cells? In **Figure 3**, bovine chips from young calves were incubated for 14 days in growth medium, which were analysed for osteocalcin, IL-10, TGFβ, OPG and RANKL (osteoblast and osteoclast markers), respectively. For detailed summary of results, see **Figure 3**.

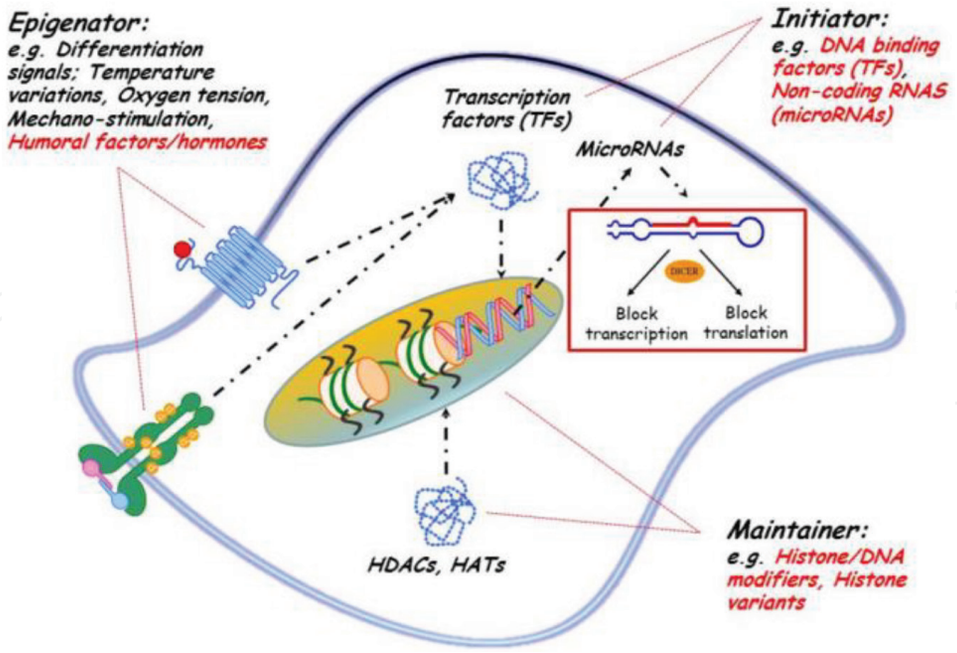


Figure 1. The epigenator-initiator-maintainer model of hormonal impact on the phenotype of a given cell. The hormone (binding to a given receptor—here represented by two different transmembrane proteins) will eventually elicit a response determined by the joint effect of transcription factors and microRNAs determining the end-point effect of the epigenator, such as cell differentiation and/or efflux of secretory products.

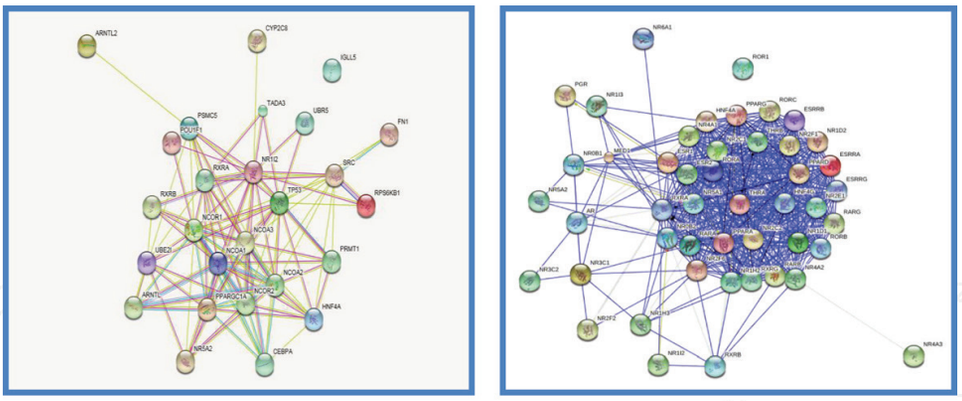


Figure 2. (a) and (b) GeneCards-based emulation of molecules interacting with NR1/NR2 (which is identical to SXR, identified/described by the Japanese researcher, Professor Satoshi Inoue). Interestingly, NR1/NR2 is integrated in a large network of interacting molecules (genes), representing various classes of transcription factors (e.g. PPARG, RORC, RARA, RXR) and not to forget the thyroid receptors A and B!

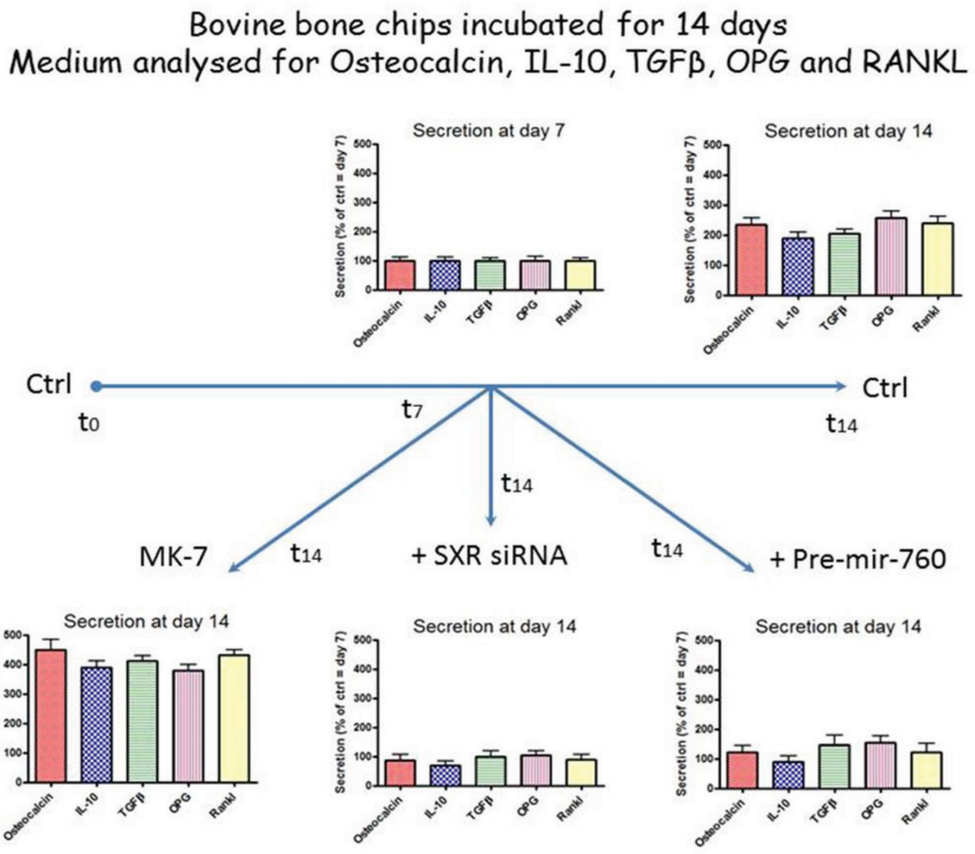


Figure 3. Bovine chips from young calves incubated as stated above. The chips were (a) incubated for 7 or 21 days and analysed for osteocalcin, IL-10, TGFβ, OPG and RANKL (all parameters' characteristics for the osteoblast phenotype); (b) incubated for 7 days and thereafter for 14 days in the presence of either vitamin K2 = MK-7; siRNA against the vitamin K2 receptor SXR or with pre-mir-760. The final measurements of secretory products were as indicated above (osteocalcin, IL-10, TGFβ, OPG and RANKL).

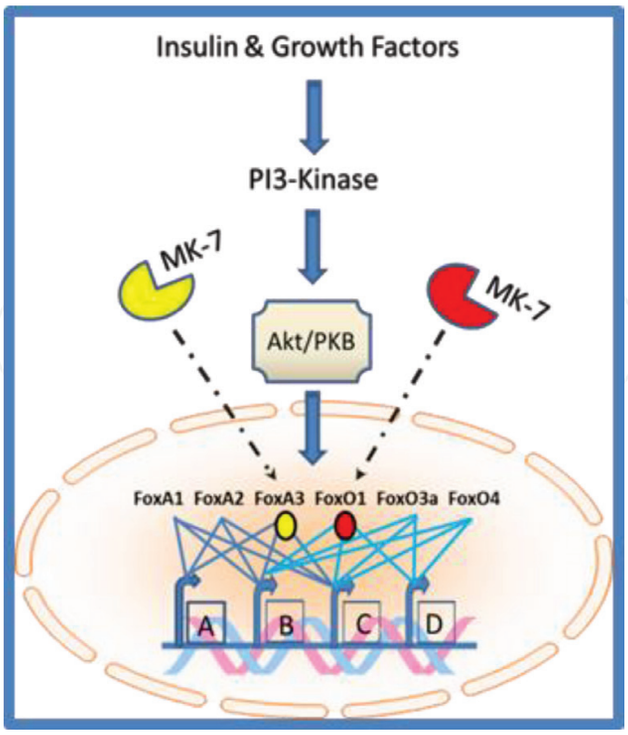


Figure 4. The impact of insulin and growth factors (GFs) on members of the FoxA and FoxO families of insulin and growth factors.

The interpretation of the experiment with 'live' bovine bone chips is the following: since MK-7 (a vitamin K2 analogue) is stimulated, while SXR siRNA and pre-mir-760 markedly reduced the secretory profile of the bovine osteoblast, it was concluded that the effect of vitamin K2 (MK-7) on the osteoblast profile of secretory molecules was stimulatory and that the effect of vitamin K2 was mediated solely through the action of the nuclear receptor SXR.

Furthermore, it was shown that one well-known and well-represented vitamin K2 'analogue' (MK-7) was able to activate (upregulate) some of the members of the FoxA and FoxO family of transcription factors. In our hands, vitamin K2 analogue MK-7 (and to a lesser extent MK-4) was able to substitute for insulin, as well as some growth factors (like growth hormone and a few interleukins (not shown)) (**Figure 4**).

Figure 5 shows a strong relationship (network between a human osteoblast microRNA profile, respectively, and a well-known plethora of marker genes) which has previously been listed according to their sequential appearance as the osteoblast develops from a stem cell into a 'full-blown', mature mineralizing osteoblast. By adding a plethora of transcription factors, sirtuins (SIRTs), histone deacetylases (HDACs) and transcription factors (with special references to the osteoblastic phenotype), a distinct pattern of interaction appeared. By adding vitamin K2, i.e. MK-7 and/or SXR, to the system, a similar pattern appeared (not shown[1]). This was also the case for other tissues as well, e.g. lung, heart, brain, muscle, white/beige fat tissues and many others (not mentioned here[2]).

[1]Details not included—patents pending.
[2]Details not included—patents pending.

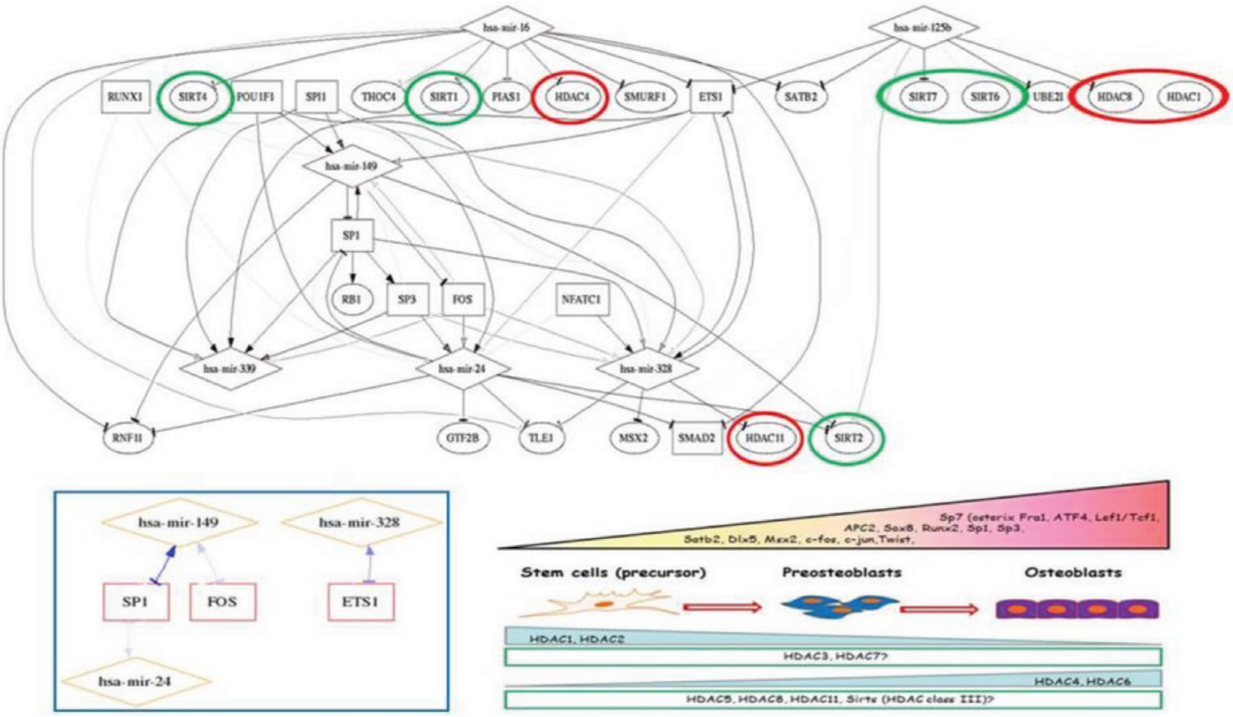


Figure 5. Network (based on the computerprogram Mir@nt@n) postulating the interaction between key microRNAs, transcription factors, sirtuins, and histone deacetylases (HDACs) in the developing osteoblast from stem cells via preosteoblasts. The compilation of genes was based on various searches on PubMed for genes appearing during the course of preosteoblast to mature (mineralizing) osteoblasts from mammalian species, including humans.

Author details

Jan Oxholm Gordeladze

Address all correspondence to: j.o.gordeladze@medisin.uio.no

Department of Molecular Medicine, Section for Biochemistry, Institute of Basic Medical Science, Blindern, Norway

References

[1] Sultana H, Watanabe K, Md MR, Takashima R, Ai O, Komai M, et al. Effects of Vitamin K2 on the Expression of Genes Involved in Bile Acid Synthesis and Glucose Homeostasis in Mice with Humanized PXR. Sendai, Japan: Laboratory of Nutrition, Graduate School of Agricultural Science, Tohoku University. 2018

[2] Ichikawa T, Horie-Inoue K, Ikeda K, Blumberg B, Inoue S. Vitamin K2 induces phosphorylation of protein kinase A and expression of novel target genes in osteoblastic cells. Journal of Molecular Endocrinology. 2007;**39**(4):239-247

[3] Mosos I. Review article—Crosstalk between vitamins A, B12, D, K, C, and E status and arterial stiffness. Disease Markers. 2017;**2017**:14. Article ID: 8784971. DOI: 10.1155/2017/8784971

[4] https://www.bodyscience.com.au/blog/dr-hogne-vik-vitamin-k2-interview/

[5] Gordeladze JO. General Reading on Vitamin K2: Vitamin K2—Vital for Health and Well-Being. IntechOpen. 2017. DOI: 10.5772/61430

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