Stem Cell Reviews and Reports (2020) 16:1005–1012
https://doi.org/10.1007/s12015-020-10009-6

Pulsed Electromagnetic Fields Modulate miRNAs During Osteogenic Differentiation of Bone Mesenchymal Stem Cells: a Possible Role in the Osteogenic-angiogenic Coupling

Monica De Mattei[1] · Silvia Grassilli[2,3] · Agnese Pellati[2] · Federica Brugnoli[2] · Elena De Marchi[1] · Deyanira Contartese[1,4] · Valeria Bertagnolo[2]

Published online: 18 July 2020

Abstract

Despite the high intrinsic ability of bone tissue to regenerate, bone healing fails in some pathological conditions and especially in the presence of large defects. Due to the strong relationship between bone development and vascularization during *in vivo* bone formation and repair, strategies promoting the osteogenic-angiogenic coupling are crucial for regenerative medicine. Increasing evidence shows that miRNAs play important roles in controlling osteogenesis and bone vascularization and are important tool in medical research although their clinical use still needs to optimize miRNA stability and delivery. Pulsed electromagnetic fields (PEMFs) have been successfully used to enhance bone repair and their clinical activity has been associated to their ability to promote the osteogenic differentiation of human mesenchymal stem cells (hMSCs). In this study we investigated the potential ability of PEMF exposure to modulate selected miRNAs involved in the osteogenic differentiation of human bone mesenchymal stem cells (hBMSCs). We show that, during *in vitro* hBMSC differentiation, PEMFs up-modulate the expression of miR-26a and miR-29b, which favor osteogenic differentiation, and decrease miR-125b which acts as an inhibitor miRNA. As PEMFs promote the expression and release of miRNAs also involved in angiogenesis, we conclude that PEMFs may represent a noninvasive and safe strategy to modulate miRNAs with relevant roles in bone repair and with the potential to regulate the osteogenic-angiogenic coupling.

Keywords Bone repair · Osteogenic differentiation · Human bone mesenchymal stem cells (hBMSCs) · Pulsed electromagnetic field (PEMF) · miRNAs · Osteogenic-angiogenic coupling

Introduction

Bone repair is a complex multistep process which involves differentiation of mesenchymal stem cells (MSCs) into osteoblasts and requires coordinated coupling between osteogenesis and angiogenesis [1, 2]. Increasing body of evidence supports the crucial role of microRNAs (miRNAs) in bone development and homeostasis [3, 4] and in promoting MSC osteogenic differentiation [5, 6]. Complex interactions among miRNAs coordinate osteogenesis and angiogenesis during development, remodeling and regeneration of the skeletal system [7].

The strategies improving bone regeneration include electromagnetic field (EMF) exposure that, in the last decades, has been applied with beneficial effects in skeletal diseases and ununited fractures [8, 9]. It is widely accepted that EMFs regulate all cells involved in the bone repair processes, including MSCs and osteoblasts [10–13] and animal studies suggest that EMFs promotes the osteogenesis-angiogenesis coupling [8].

EMFs were reported to modulate different phases of osteogenic differentiation involving a number of intracellular signaling pathways [8]. We previously demonstrated that a pulsed electromagnetic field (PEMF) with specific physical parameters stimulates the osteoblast differentiation of human bone mesenchymal stem cells (hBMSCs) also by increasing

✉ Monica De Mattei
monica.demattei@unife.it

✉ Valeria Bertagnolo
bgv@unife.it

[1] Department of Medical Sciences, University of Ferrara, Ferrara, Italy

[2] Department of Morphology, Surgery and Experimental Medicine, University of Ferrara, Ferrara, Italy

[3] LTTA Centre, University of Ferrara, Ferrara, Italy

[4] Laboratory Preclinical and Surgical Studies, IRCCS-Istituto Ortopedico Rizzoli, Bologna, Italy

the expression of crucial osteogenic transcription factors [12, 13], indicative of a PEMF induced modulation of gene expression. The expression of miRNAs, targeting inhibitors of the major osteogenic transcription factors [14], was recently correlated with EMF activity in neural cells [15] but, to our knowledge, only miR-21 was associated with osteogenic differentiation of human MSCs induced by PEMFs [16].

In this study, the role of PEMFs in modulating miRNAs that have been previously associated to osteogenic and/or angiogenic differentiation was investigated in differentiating hBMSCs, revealing that PEMFs regulate miRNAs involved in different phases of osteogenic differentiation and bone repair, including angiogenesis.

Materials and Methods

BMSC Osteogenic Differentiation and PEMF Exposure

Human bone marrow mesenchymal stem cells (hBMSCs) (Lonza, Walkersville, MD) were cultured at the density of 5,000 cells/cm^2 in complete mesenchymal stem cell basal medium (MSCBM) (Lonza), in a humidified atmosphere containing 5% CO_2 at 37 °C. At the third passage cells were induced to osteogenic differentiation by culture in the Osteogenic Differentiation Medium (OM) (Lonza) for 28 days.

To assess the effects of PEMFs, hBMSCs cultured in OM were subjected to PEMF exposure as reported in previous studies [12, 13]. In particular, the used power generator (IGEA S.p.A., Carpi, Italy) produced a pulsed signal with pulse duration of 1.3 ms and frequency of 75 Hz, yielding a duty cycle of 1/10. The intensity peak of the magnetic field was 1.5 mT and the exposure was maintained continuously for the whole differentiation time. Unexposed cells were grown in the same incubator, placed at a distance from the coils where no difference from background magnetic field was observed when the PEMF generator was turned on. In all the experiments, the medium was changed twice a week.

At selected differentiation times, cells were harvested for analysis of osteogenic and angiogenic markers and of miRNAs.

Evaluation of Osteogenic Differentiation

Detection of osteocalcin (OC) levels, calculated as ng OC/μg DNA, was performed by using a commercial ELISA kit (Invitrogen, Rockville, MD). Mineralization was visualized by staining formalin fixed cells with 2% Alizarin Red S (ARS) for 5 min and images were taken using a standard light microscope (Nikon Eclipse TE 2000-E microscope, Nikon Instruments Spa, Sesto Fiorentino, Italy) equipped with a digital camera (DXM 1200F; Nikon Instruments Spa, Italy).

Quantitative Real-Time PCR Assays

High-quality RNA from hBMSCs cells under different experimental conditions was extracted with miRNeasy micro kit (Qiagen S.p.A, Milan, I) and RNA was subjected to single-stranded cDNA synthesis by using the TaqMan MicroRNA RT kit (Life Technologies, Monza, I). The obtained cDNAs were employed as templates for quantitative Real-Time PCR-based miR-26a, miR-29b, miR-125b, miR-210 and miR-218 expression measurements using TaqMan MicroRNA Assays (Life Technologies), as previously reported [17, 18].

For RUNX-2, vascular endothelial growth factor (VEGF) and von Willebrand factor (VWF) analysis, RNA was reverse transcribed with High-Capacity cDNA Reverse Transcription Kit (Life Technologies) and qPCR was performed using the iTaq Universal SYBR green SuperMix (Bio-Rad Laboratories, Hercules, CA) on a QuantStudio™ 5 Real-Time PCR System (Life Technologies), following manufacturer's instruction.

The primers used were as follows:
RUNX-2) Fw: AAGCTTGATGACTCTAAACC; Rev: TCTGTAATCTGACTCTGTCC,
VEGF) Fw: TCGGGCCTCCGAAACCATGA; Rev: CCTGGTGAGAGATCTGGTTC;
VWF) Fw: CCTGTGAGTCCATTGGGGAC; Rev: AGTCTTCAGGGTCAACGCAG.

Culture Media Analysis

After 21 days, culture media from cells under our different experimental conditions were collected and centrifuged at 14,000 g for 10 min at RT to remove cell debris.

VEGF levels were assessed by PicoKine™ ELISA kit (Boster, distributed by Tema Ricerca, Bologna, Italy) as previously described [19]. Briefly, 50 μl of cell supernatant were diluted (1:1) in sample diluent buffer and the ELISA assay was performed according to manufacturers' instructions. All specific culture media were loaded as appropriate blank controls. Data were normalized for the total number of cells present in culture wells.

Isolation of miRNAs was performed using miRNeasy Serum/Plasma (Qiagen), essentially following manufacturer's instruction and total RNA was eluted in 30 μl of RNase-free water. RNA was subjected to single-stranded cDNA synthesis by using the TaqMan MicroRNA RT kit (Life Technologies) and the obtained cDNAs were employed as templates for quantitative Real-Time PCR of miR-26a, miR-29b, miR-125b, miR-210 and miR-218 using TaqMan MicroRNA Assays (Life Technologies), as previously reported [17, 18].

Statistical Analysis

Statistical analysis was performed using the 2-tailed Student's t test for unpaired data with the GraphPad Prism 6.0 statistical package (GraphPad Software, San Diego, CA). P values < 0.05 were considered statistically significant.

Results and Discussion

Repair of bone defects represents a major challenge for clinicians, especially when large bone faults are present [2, 9]. Because of the increasing incidence for bone disorders such as fractures and osteoporosis, there is an urgent need for therapies improving the efficiency of bone repair. Among the most recent strategies hypothesized to increase bone regeneration, the use of miRNAs is attracting increasing attention. In fact, *in vitro* and *in vivo* studies have shown that complex interactions among miRNAs affect bone development and homeostasis [3], as well as the coordinated interaction between osteogenesis and angiogenesis which occurs during development, remodeling and regeneration of the skeletal system [7]. Despite emerging evidence reveals the potential advantage of the application of miRNAs in the treatment of bone fracture, osteoporosis, and other bone-related diseases [20], miRNA-targeting therapy is still in development due to some crucial issues, including how to optimize miRNA stability and delivery. In this context, we investigated here the potential role of PEMFs, a noninvasive and safe physical strategy to accelerate bone repair, in modulating miRNAs involved in different steps of osteogenic differentiation. As it is well established that PEMF stimulation promotes osteogenesis and angiogenesis in an orchestrated spatiotemporal manner, ultimately enhancing the self-repair capability of bone tissue [8], among the miRNA investigated we included miRNAs involved in both osteogenesis and angiogenesis. The PEMF effects on miRNAs were evaluated in hBMSCs induced to osteogenic differentiation, as these cells are mainly involved in *in vivo* bone repair and represent an important source of osteogenic cells in tissue engineering, with good prospects for applications in the field of bone defect repair and regeneration [21].

In agreement with our previous studies [12, 13], PEMF exposure accelerates differentiation of hBMSCs grown in osteogenic medium (OM), as shown by the evaluation of mineralization (Fig. 1a), RUNX-2 expression and OC levels (Fig. 1b). On the basis of the suggested role of EMFs in promoting the osteogenesis-angiogenesis coupling [8], we also investigated the expression of VEGF, the master regulator of vascular growth, known to increase during osteogenic differentiation [22]. As shown in Fig. 1b and 21 days of PEMF exposure increased the expression of VEGF in differentiating cells, in parallel with the increase in OC level and mineralization. The analysis of VWF, used to assess endothelial differentiation of MSCs [23] failed to show expression of this angiogenic marker in all the explored experimental conditions (data not shown), allowing to conclude that PEMFs does not induce efficient endothelial differentiation of hBMSCs grown in osteogenic differentiation medium.

We then investigated five miRNAs selected on the basis of their known roles in different stages of the osteogenic differentiation and/or in osteogenic-angiogenic coupling. The analysis was performed after 3 days of culture, at which we found that PEMFs strongly induced RUNX-2 (Fig. 1b) and after 21 days of treatment, corresponding to significant increases induced by PEMFs on late osteogenic markers [13], including osteocalcin (OC), and on VEGF expression (Fig. 1b).

We firstly evaluated the effects of PEMF on miR-26a and miR-29b, both known to promote osteogenesis by targeting osteo-inhibitory proteins [24]. MiR-26a, that appears upregulated in newly-formed bone, promotes hBMSCs osteogenic differentiation by targeting GSK3β [25] and has been suggested for miRNA-based strategies to improve tissue regeneration [1]. Despite we failed to show modifications of miR-26a in differentiating hBMSCs with respect to cells grown in control conditions (Fig. 2a), we revealed that this miRNA is induced by PEMFs at both the analyzed exposure times (Fig. 2a and b). On the basis of the reported role of miR-26a in inducing early (RUNX-2) and late (OC) osteogenic markers as well as VEGF [1, 26], our results suggest that PEMF exposure has the potential for coupling angiogenesis to the osteogenic differentiation of hBMSCs.

We then evaluated le levels of miR-29b that regulates osteogenic differentiation via several mechanisms and was previously used to develop new systems to promote osteogenic differentiation by deliver miRNAs into BMSCs cytoplasm [27, 28]. On one hand, miR-29b targets inhibitors of the Wnt, ERK and MAPK signaling pathways at early stages of osteogenesis; on the other hand, miR-29b directly downregulates osteogenic differentiation suppressors such as histone deacetylase-4 (HDAC4), transforming growth factor3, CTNNBIP1, and DUSP2 [28]. MiR-29b seems also regulate the late differentiation steps by suppressing synthesis of extracellular matrix proteins, relevant to preserve the differentiated phenotype during mineralization of mature osteoblasts [7]. We revealed here that miR-29b level increases during osteogenic differentiation of hBMSCs (Fig. 2a), and that 21 days of PEMF exposure further up-modulates the expression of this miRNA (Fig. 2b). This first bulk of data suggests that the PEMF induced increase of osteogenic differentiation may be correlated with up-modulation of miRNAs involved in both early and late stages of osteoblast maturation. As nor miR-26a neither miR-29b are reported to directly target osteogenic transcription factors, but both are known to down-modulate RUNX-2 repressors [29, 30], our data support the assumption that PEMFs promote osteogenic differentiation also by the removal of osteogenic inhibitors.

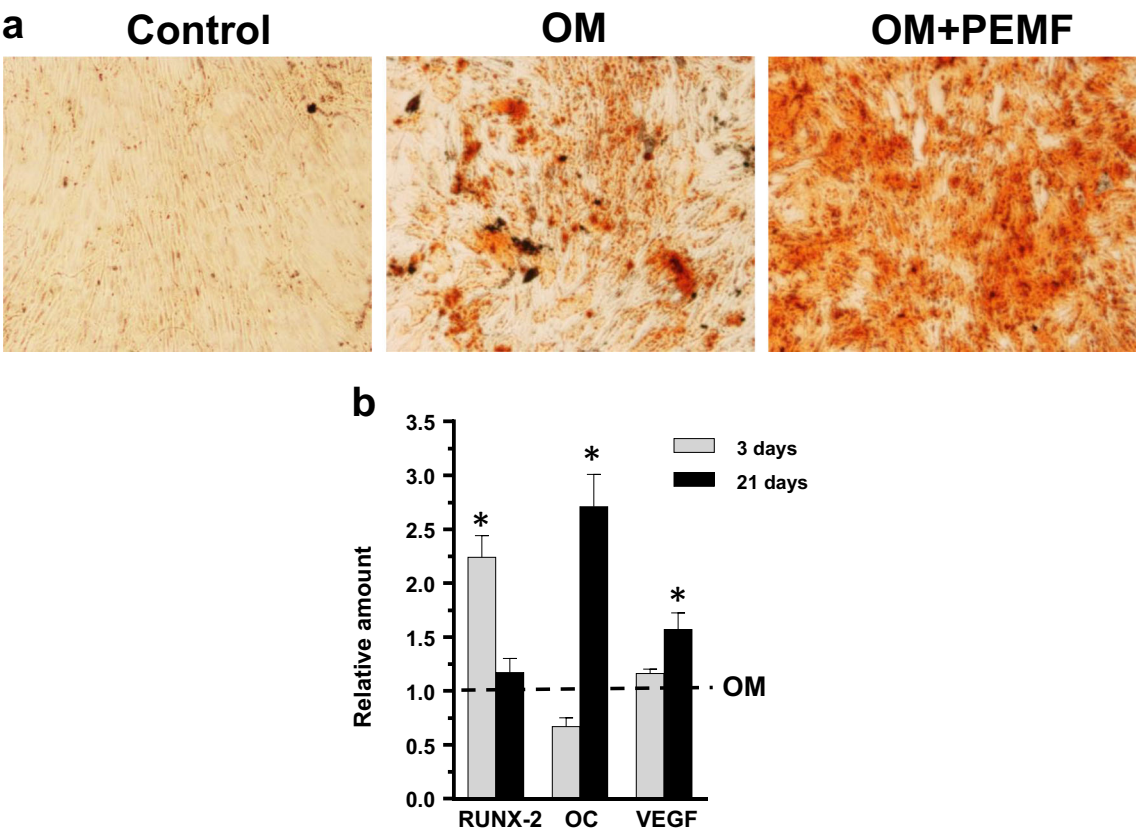

Fig. 1 Effects of PEMFs on osteogenic differentiation of hBMSCs. In **a**, representative matrix mineralization evaluated by Alizarin red staining of hBMSCs after 28 days of culture in control conditions, in osteogenic differentiating medium (OM) and in osteogenic differentiating medium with continuous PEMFs exposure (OM + PEMF). In **b**, expression of Runx2, OC and VEGF after 3 and 21 days of osteogenic differentiation. All the data are the mean of 3 separate experiments ± SD. *P < 0.05 compared to cell grown in differentiation medium (OM), taken as 1

We furthermore investigated the effects of PEMFs on miR-125b, which negatively regulates the osteogenic differentiation of hBMSCs cells by directly targeting BMPR1b, a member of the bone morphogenetic protein (BMP) receptor family [31, 32]. As silencing of miR-125b increased the mRNA levels of osteoblastic marker genes ALP, OC, and OPN, RUNX-2 is considered as an indirect target of miR-125b as well [31]. Expression of miR-125b was also correlated with reduced *in vitro* tube formation by endothelial cells grown under low oxygenation, suggesting for this miRNA a role in hypoxic regulation of angiogenesis [7]. At variance with the expected low miR-125b levels during osteogenic differentiation of hBMSCs [31], we found that this miRNA increased after 21 days of culture in differentiating medium (Fig. 2a). However, this result is in agreement with a previous study on the same cell model [33] and on periodontal cells [34] indicating a main role of miR-125b in driving the first rather than the late phases of differentiation. Interestingly, 21 days PEMF exposure significantly decreased miR-125b levels in differentiating cells (Fig. 2b). As the *in vitro* inhibition of miR-125b enhances the osteogenic differentiation of hBMSCs [32], and its down-modulation *in vivo* promotes the new bone formation on a scaffold and the repair of the bone defects [31], our data suggest that PEMFs may promote osteogenic differentiation also by down-modulating inhibitor miRNAs.

Moreover, we investigated the role of PEMFs in modulating miR-210, a hypoxia-inducible miRNA that acts as a positive regulator of osteoblastic differentiation *in vitro* by up-regulating the expression of multiple key osteogenic genes [35]. Taking into account that oxygenation lower than 1% is an angiogenic stimulus in bone formation [36], and that in rat BMSCs, miR-210 promoted the expression of VEGF [37], miR-210 may be considered at the interface between osteogenesis and angiogenesis [7]. We revealed here that, in hBMSCs induced to osteogenic differentiation, miR-210 is down-regulated with respect to undifferentiated cells (Fig. 2a). As miR-210 is modulated by oxygen availability, it is reasonable to conclude that, in our differentiation model, oxygenation is guarantee and cannot constitute an angiogenic stimulus. Interestingly, 21 days of PEMF exposure during differentiation of hBMSCs induced a slight but significant increase of miR-210 (Fig. 2b), suggestive of hypoxia-independent roles of PEMFs in up-modulating this osteogenesis-angiogenesis coupled miRNA.

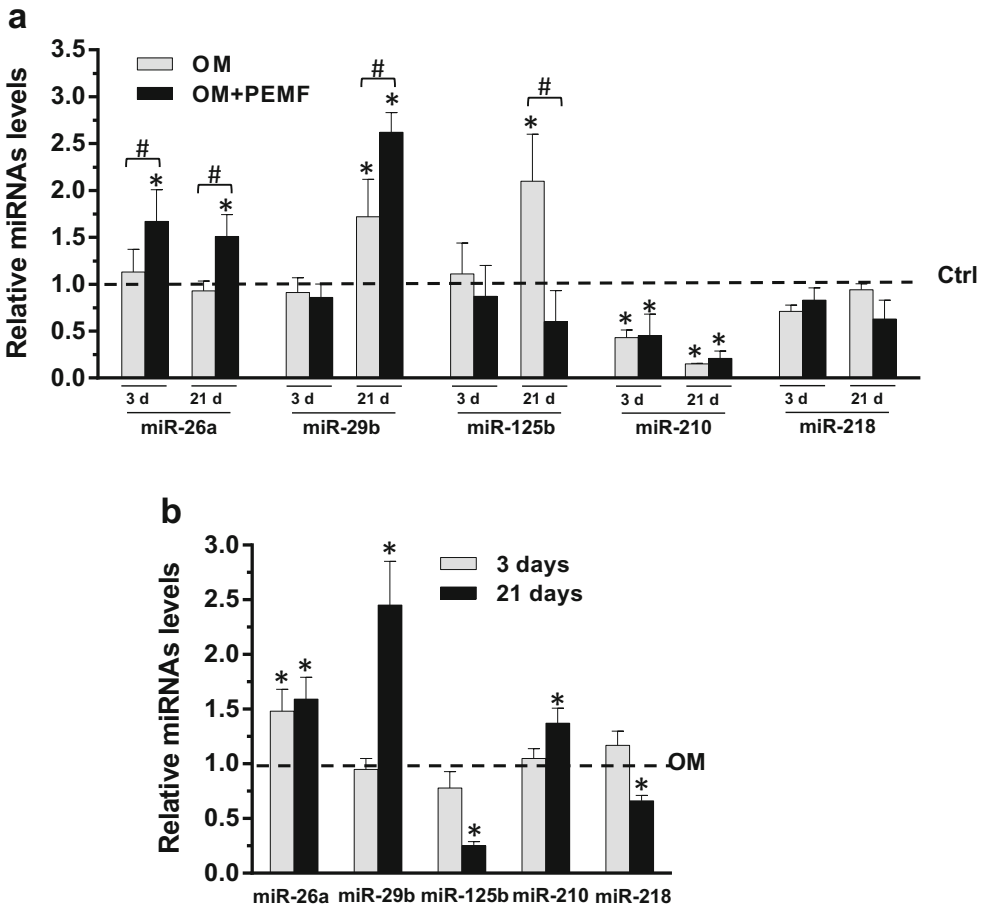

Fig. 2 Effects of PEMFs on miRNAs expression in hBMSCs cells induced to osteogenic differentiation. In **a**, QRT-PCR analysis of miR-26a, miR-29b, miR-125b, miR-210 and miR-218 in hBMSCs cells cultured in osteogenic differentiating medium (OM), with or without PEMF exposure, for 3 and 21 days. Transcripts levels are shown as fold changes relative to the control condition (Ctrl), taken as 1 and indicated with a dotted line, by using the $2^{-\Delta\Delta Ct}$ method. The data are the mean of 3 separate experiments ± SD. $^{*}P < 0.05$ compared to control. $^{\#}P < 0.05$ between bars. In **b**, levels of the miRNAs in differentiating hBMSCs exposed to PEMFs with respect to hBMSCs growing in differentiation medium, taken as 1 and indicated with a dotted line. The data are the mean of 3 separate experiments ± SD.$^{*}P < 0.05$

Finally, we assessed the potential role of PEMFs in modulating miR-218, which promotes osteogenic differentiation *in vitro* and resulted highly expressed in exosomes from hBMSCs induced to osteogenic differentiation [38]. At variance with mouse BMSCs, in which osteogenic differentiation induces miR-218 expression [39], we failed to reveal effects of OM on miR-218 levels in hBMSCs (Fig. 2a). This inconsistency, on one hand, may be ascribed to the precursor-related modulation of miR-218 during *in vitro* differentiation, on the other hand, to the elusive *in vivo* role of miR-218 in humans [39]. Nevertheless, 21 days of PEMF exposure induced a slight but significant decrease of miR-218 in hBMSCs differentiating cells (Fig. 2b), compatible with the PEMF induced release of miR-218 outside the cell, as shown in Fig. 3a.

In fact, to verify if PEMFs could also modulate the release of the miRNA investigated, we evaluated their levels in culture media. As reported in Fig. 3a, in addition to miR-218, PEMFs induced the release of miR-26a and miR-210, involved in both osteogenesis and angiogenesis, as previously reported [26, 37, 40]. Also the level of VEGF was slightly increased in the culture medium of PEMF exposed hBMSCs. These results suggests the existence of autocrine and paracrine ways by means PEMFs can regulate both osteogenesis and angiogenesis, in line with the role of PEMFs stimulation described in other models [8].

Conclusions

Emerging evidence shows that miRNAs represent attractive candidates to promote bone repair in regenerative medicine. In this study we demonstrate that PEMFs modulate miRNAs expression during hBMSCs osteogenic differentiation. In particular, we found that PEMFs with specific physical parameters regulate the expression and the release of miRNAs potentially involved in both osteogenesis and angiogenesis, which are highly coordinated events during *in vivo* bone repair. Although further extensive studies are required to elucidate the entire pool of miRNAs modulated by PEMF and their related molecular networks, our data suggest that PEMF exposure represents a non-invasive and safe strategy to *in vivo* modulate miRNAs, avoiding the limitations of direct miRNA delivery.

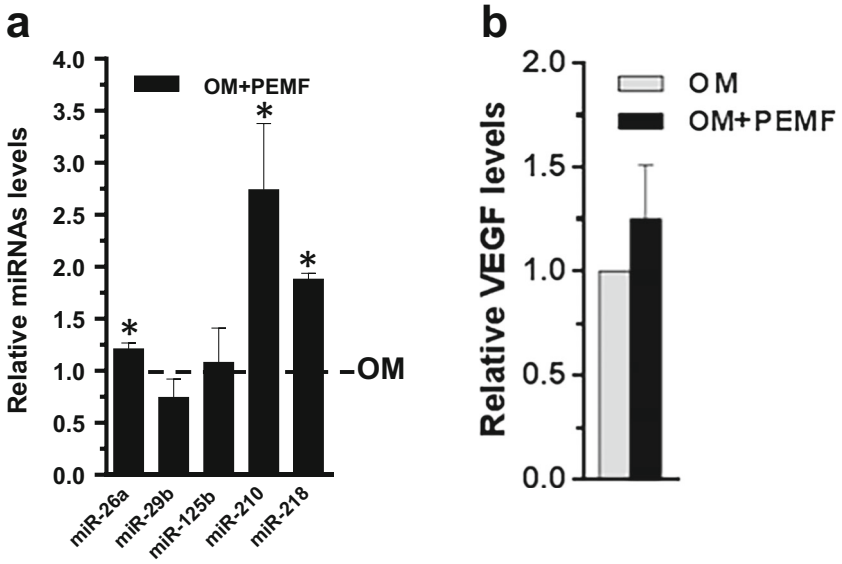

Fig. 3 Effects of PEMFs on miRNAs levels in culture medium from hBMSCs cells induced to osteogenic differentiation. In **a**, QRT-PCR analysis of miR-26a, miR-29b, miR-125b, miR-210 and miR-218 in culture medium from 21 days differentiating hBMSCs exposed to PEMFs with respect to hBMSCs growing in differentiation medium (OM). Transcripts levels are shown as fold changes relative to the differentiating condition (OM), taken as 1 and indicated with a dotted line, by using the $2^{-\Delta\Delta Ct}$ method. In **b**, VEGF levels in culture medium from hBMSCs cells cultured for in the same experimental conditions. The data are the mean of 3 separate experiments ± SD.*$P < 0.05$ compared to the OM condition

Acknowledgements We thank Dr. Mattia Ferrarese, Department of Life Sciences and Biotechnology, University of Ferrara, for providing primers for VWF analysis.

Authors' Contribution MDM and VB was responsible for the study concept, supervised all the experiments and integrated the results; SG, AP, FB, EDM and DC performed experiments and prepared figures; VB and MDM drafted the manuscript with input and approval from all authors.

Funding Information This study was funded by grants from by IGEA S.p.A (Carpi, Italy) to MDM and from University of Ferrara to MDM and VB.

Data Availability All data generated or analyzed during this study are included in this published article.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethics Approval Not applicable.

Consent Not applicable.

References

1. Li, Y., Fan, L., Liu, S., Liu, W., Zhang, H., Zhou, T., Wu, D., Yang, P., Shen, L., Chen, J., & Jin, Y. (2013). The promotion of bone regeneration through positive regulation of angiogenic-osteogenic coupling using microRNA-26a. *Biomaterials, 34*, 5048–5058.
2. Ho-Shui-Ling, A., Bolander, J., Rustom, L. E., Johnson, A. W., Luyten, F. P., & Picart, C. (2018). Bone regeneration strategies: Engineered scaffolds, bioactive molecules and stem cells current stage and future perspectives. *Biomaterials, 80*, 143–162.
3. Valenti, M. T., Carbonare, D., L., & Mottes, M. (2018). Role of microRNAs in progenitor cell commitment and osteogenic differentiation in health and disease. *International Journal of Molecular Medicine, 41*, 2441–2449.
4. Li, B. (2018). MicroRNA Regulation in Osteogenic and Adipogenic Differentiation of Bone Mesenchymal Stem Cells and its Application in Bone Regeneration. *Current Stem Cell Research & Therapy, 13*, 26–30.
5. Huang, C., Geng, J., & Jiang, S. (2017). MicroRNAs in regulation of osteogenic differentiation of mesenchymal stem cells. *Cell and Tissue Research, 368*, 229–238.
6. Peng, S., Gao, D., Gao, C., Wei, P., Niu, M., & Shuai, C. (2016). MicroRNAs regulate signaling pathways in osteogenic differentiation of mesenchymal stem cells (Review). *Molecular Medicine Reports, 14*, 623–629.
7. Fröhlich, L. F. (2019). Micrornas at the Interface between Osteogenesis and Angiogenesis as Targets for Bone Regeneration. *Cells*, *8*, E121.
8. Yuan, J., Xin, F., & Jiang, W. (2018). Underlying Signaling Pathways and Therapeutic Applications of Pulsed Electromagnetic Fields in Bone Repair. *Cellular Physiology and Biochemistry, 46*, 1581–1594.
9. Massari, L., Benazzo, F., Falez, F., Perugia, D., Pietrogrande, L., Setti, S., Osti, R., Vaienti, E., Ruosi, C., & Cadossi, R. (2019). Biophysical stimulation of bone and cartilage: state of the art and future perspectives. *International Orthopaedics, 43*, 539–551.
10. De Mattei, M., Gagliano, N., Moscheni, C., Dellavia, C., Calastrini, C., Pellati, A., Gioia, M., Caruso, A., & Stabellini, G. (2005). Changes in polyamines, c-myc and c-fos gene expression in osteoblast-like cells exposed to pulsed electromagnetic fields. *Bioelectromagnetics, 26*, 207–214.
11. Lohmann, C. H., Schwartz, Z., Liu, Y., Li, Z., Simon, B. J., Sylvia, V. L., Dean, D. D., Bonewald, L. F., Donahue, H. J., & Boyan, B. D. (2003). Pulsed electromagnetic fields affect phenotype and connexin 43 protein expression in MLO-Y4 osteocyte-like cells

and ROS 17/2.8 osteoblast-like cells. *Journal of Orthopaedics Research, 21*, 326–334.

12. Ongaro, A., Pellati, A., Bagheri, L., Fortini, C., Setti, S., & De Mattei, M. (2014). Pulsed electromagnetic fields stimulate osteogenic differentiation in human bone marrow and adipose tissue derived mesenchymal stem cells. *Bioelectromagnetics, 35*, 426–436.
13. Bagheri, L., Pellati, A., Rizzo, P., Aquila, G., Massari, L., De Mattei, M., & Ongaro, A. (2018). Notch pathway is active during osteogenic differentiation of human bone marrow mesenchymal stem cells induced by pulsed electromagnetic fields. *Journal of Tissue Engineering and Regenerative Medicine, 12*, 304–315.
14. Wang, J., Liu, S., Li, J., Zhao, S., & Yi, Z. (2019). Roles for miRNAs in osteogenic differentiation of bone marrow mesenchymal stem cells. *Stem Cell Research and Therapy, 10*, 197.
15. Leone, L., Podda, M. V., & Grassi, C. (2015). Impact of electromagnetic fields on stem cells: common mechanisms at the crossroad between adult neurogenesis and osteogenesis. *Frontiers in Cellular Neuroscience, 9*, 228.
16. Selvamurugan, N., He, Z., Rifkin, D., Dabovic, B., & Partridge, N. C. (2017). Pulsed Electromagnetic Field Regulates MicroRNA 21 Expression to Activate TGF-β Signaling in Human Bone Marrow Stromal Cells to Enhance Osteoblast Differentiation. *Stem Cells International*, *2017*, 2450327.
17. Vezzali, F., Grassilli, S., Lambertini, E., Brugnoli, F., Patergnani, S., Nika, E., Piva, R., Pinton, P., Capitani, S., & Bertagnolo, V. (2018). Vav1 is necessary for PU.1 mediated upmodulation of miR-29b in acute myeloid leukaemia-derived cells. *Journal of Cellular and Molecular Medicine, 22*, 3149–3158.
18. Volinia, S., Bertagnolo, V., Grassilli, S., Brugnoli, F., Manfrini, M., Galasso, M., Scatena, C., Mazzanti, C. M., Lessi, F., Naccarato, G., Caligo, A., Bianchini, E., Piubello, Q., Orvieto, E., Rugge, M., Natali, C., Reale, D., Vecchione, A., Warner, S., Croce, C. M., & Capitani, S. (2018). Levels of miR-126 and miR-218 are elevated in ductal carcinoma in situ (DCIS) and inhibit malignant potential of DCIS derived cells. *Oncotarget, 9*, 23543–23553.
19. Pegoraro, A., Bortolotti, D., Marci, R., Caselli, E., Falzoni, S., De Marchi, E., Di Virgilio, F., Rizzo, R., & Adinolfi, E. (2020). The P2 × 7 Receptor 489C > T Gain of Function Polymorphism Favors HHV-6A Infection and Associates With Female Idiopathic Infertility. *Frontiers in Pharmacology, 11*, 96.
20. Sun, X., Guo, Q., Wei, W., Robertson, S., Yuan, Y., & Luo, X. (2019). Current Progress on MicroRNA-Based Gene Delivery in the Treatment of Osteoporosis and Osteoporotic Fracture. *International Journal of Endocrinology*, 6782653.
21. Oryan, A., Kamali, A., Moshiri, A., & Baghaban Eslaminejad, M. (2017). Role of Mesenchymal Stem Cells in Bone Regenerative Medicine: What Is the Evidence? *Cells Tissues and Organs, 204*, 59–83.
22. Chen, J., Deng, L., Porter, C., Alexander, G., Patel, D., Vines, J., Zhang, X., Chasteen-Boyd, D., Sung, H. J., Li, Y. P., Javed, A., Gilbert, S., Cheon, K., & Jun, H. W. (2018). Angiogenic and Osteogenic Synergy of Human Mesenchymal Stem Cells and Human Umbilical Vein Endothelial Cells Cocultured on a Nanomatrix. *Scientific Reports, 8*, 15749.
23. Wang, C., Li, Y., Yang, M., Zou, Y., Liu, H., Liang, Z., Yin, Y., Niu, G., Yan, Z., & Zhang, B. (2018). Efficient Differentiation of Bone Marrow Mesenchymal Stem Cells into Endothelial Cells in Vitro. *European Journal of Vascular and Endovascular Surgery, 55*, 257e265.
24. Trompeter, H. I., Dreesen, J., Hermann, E., Iwaniuk, K. M., Hafner, M., Renwick, N., Tuschl, T., & Wernet, P. (2013). MicroRNAs miR-26a, miR-26b, and miR-29b accelerate osteogenic differentiation of unrestricted somatic stem cells from human cord blood. *BMC genomics, 14*, 111.
25. Su, X., Liao, L., Shuai, Y., Jing, H., Liu, S., Zhou, H., Liu, Y., & Jin, Y. (2015). MiR-26a functions oppositely in osteogenic differentiation of BMSCs and ADSCs depending on distinct activation and roles of Wnt and BMP signaling pathway. *Cell Death Disease, 6*, e1851.
26. Hosseinpour, S., He, Y., Nanda, A., & Ye, Q. (2019). MicroRNAs Involved in the Regulation of Angiogenesis in Bone Regeneration. *Calcified Tissue International, 105*, 223–238.
27. Suh, J. S., Lee, J. Y., Choi, Y. S., Chung, C. P., & Park, Y. J. (2013). Peptide-mediated intracellular delivery of miRNA-29b for osteogenic stem cell differentiation. *Biomaterials, 34*, 4347–4359.
28. Liu, Q., Lin, Z., Liu, Y., Du, J., Lin, H., & Wang, J. (2019). Delivery of miRNA-29b Using R9-LK15, a Novel Cell-Penetrating Peptide, Promotes Osteogenic Differentiation of Bone Mesenchymal Stem Cells. *Biomed Research International*, *2019*, 3032158.
29. Narayanan, A., Srinaath, N., Rohini, M., & Selvamurugan, N. (2019). Regulation of Runx2 by MicroRNAs in osteoblast differentiation. *Life Science, 232*, 116676.
30. Moghaddam, T., & Neshati, Z. (2019). Role of microRNAs in osteogenesis of stem cells. *Journal of Cellular Biochemistry, 120*, 14136–14155.
31. Wang, H., Xie, Z., Hou, T., Li, Z., Huang, K., Gong, J., Zhou, W., Tang, K., Xu, J., & Dong, S. (2017). MiR-125b Regulates the Osteogenic Differentiation of Human Mesenchymal Stem Cells by Targeting BMPR1b. *Cellular Physiology and Biochemistry, 41*, 530–542.
32. Chen, S., Yang, L., Jie, Q., Lin, Y. S., Meng, G. L., Fan, J. Z., Zhang, J. K., Fan, J., Luo, Z. J., & Liu, J. (2014). MicroRNA125b suppresses the proliferation and osteogenic differentiation of human bone marrowderived mesenchymal stem cells. *Molecular Medicine Reports, 9*, 1820–1826.
33. Pinto, M. T., Nicolete, L. D. F., Rodrigues, E. S., Palma, P. V. B., Orellana, M. D., Kashima, S., & Covas, D. T. (2013). Overexpression of hsa-miR-125b During Osteoblastic Differentiation Does Not Influence Levels of Runx2, Osteopontin, and ALPL Gene Expression. *Brazilian Journal of Medical and Biological Research, 46*, 676–680.
34. Xue, N., Qi, L., Zhang, G., & Zhang, Y. (2018). miRNA-125b Regulates Osteogenic Differentiation of Periodontal Ligament Cells Through NKIRAS2/NF-κB Pathway. *Cellular Physiology and Biochemistry, 48*, 1771–1781.
35. Hu, B., Li, Y., Wang, M., Zhu, Y., Zhou, Y., Sui, B., Tan, Y., Ning, Y., Wang, J., He, J., Yang, C., & Zou, D. (2018). Functional reconstruction of critical-sized load-bearing bone defects using a Sclerostin-targeting miR-210-3p-based construct to enhance osteogenic activity. *Acta Biomaterialia, 76*, 275–282.
36. Riddle, R. C., Khatri, R., Schipani, E., & Clemens, T. L. (2009). Role of hypoxia-inducible factor-1alpha in angiogenic-osteogenic coupling. *Journal of Molecular Medicine (Berl), 87*, 583–590.
37. Liu, X. D., Cai, F., Liu, L., Zhang, Y., & Yang, A. L. (2015). MicroRNA-210 is involved in the regulation of postmenopausal osteoporosis through promotion of VEGF expression and osteoblast differentiation. *Biological Chemistry, 396*, 339–347.
38. Xu, J. F., Yang, G. H., Pan, X. H., Zhang, S. J., Zhao, C., Qiu, B. S., Gu, H. F., Hong, J. F., Cao, L., Chen, Y., Xia, B., Bi, Q., & Wang, Y. P. (2014). Altered microRNA expression profile in exosomes during osteogenic differentiation of human bone marrow-derived mesenchymal stem cells. *PLoS One, 9*, e114627.
39. Shi, L., Feng, L., Liu, Y., Duan, J. Q., Lin, W. P., Zhang, J. F., & Li, G. (2018). MicroRNA-218 Promotes Osteogenic Differentiation of

Mesenchymal Stem Cells and Accelerates Bone Fracture Healing. *Calcified Tissue International, 103*, 227–236.

40. Zuo, R., Kong, L., Wang, M., Wang, W., Xu, J., Chai, Y., Guan, J., Kang, Q. (2019). Exosomes derived from human CD34 < sup>+ </sup > stem cells transfected with miR-26a prevent glucocorticoid-induced osteonecrosis of the femoral head by promoting angiogenesis and osteogenesis. *Stem Cell Research and Therapy*, *10*, 321.