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# MICRORNAS MIR-29B-3P, MIR-365A-3P, MIR-550A-3P ARE CORRELATED TO HISTOMORPHOMETRY AND BONE TURNOVER MARKERS IN IDIOPATHIC OSTEOPOROSIS

Roland Kocijan<sup>1,3</sup>, Christian Muschitz<sup>1</sup>, Fabian Plachel<sup>1</sup>, Rainer Dormann<sup>1</sup>, Elisabeth Geiger<sup>2</sup>, Susanna Skalicky<sup>2</sup>, Heinrich Resch<sup>1</sup>, Patrick Heimel<sup>3</sup>, Heinz Redl<sup>3</sup>, Astrid Fahrleitner-Pammer<sup>4</sup>, Johannes Grillari<sup>5</sup>, Matthias Hackl<sup>2,5</sup>

1 St. Vincent Hospital – Medical Department II, The VINFORCE Study Group, Academic Teaching Hospital of the Medical University of Vienna, Austria  
2 TAmiRNA GmbH, Muthgasse 18, 1190 Vienna, Austria  
3 Ludwig Boltzmann Institute for Experimental and Clinical Traumatology; Donauerschlingenstraße 13, 1200 Vienna, Austria  
4 Department of Internal Medicine, Division of Endocrinology and Metabolism, Medical University of Graz, Austria  
5 Department of Biotechnology, University of Natural Resources and Life Sciences Vienna, Austria

## BACKGROUND METHODS RESULTS CONCLUSION

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression. Several miRNAs are known to be involved in the regulation of bone homeostasis. Circulating miRNAs are actively released from donor cells in the blood or solid tissues, and have been shown to reflect the physiology of donor cells, or to modulate the behavior of their target cells. We previously reported 19 circulating miRNAs with common deregulation in serum of pre-, and postmenopausal women as well as males with idiopathic osteoporosis and low-traumatic fracture (see Poster #SU0324). Based on these findings, we tested the correlation between bone specific circulating miRNAs and bone histomorphometry, bone mineral density as well as bone turnover markers (BTM).

Transiliacal bone biopsies were performed in 36 patients (46.6±13.0 years) with idiopathic osteoporosis and low-traumatic fractures to assess bone histomorphometry (BV/TV, BS/BV, OS/BS, ES/BS, QS/BS, Tb.N, Tb.Th, MS/BS, MAR, BFR/BS). Time to last fracture was at least 6 months. Secondary causes for osteoporosis were excluded by careful clinical examination. BTM including iPTH, 25(OH)vitamin D, BALp, OC, PINP, OPG, RANKL, TRAP5b, CTX were analyzed (see table 1). RNA was extracted from serum using phenol/chloroform extraction. Reverse-transcription quantitative PCR (RT-qPCR) analysis of circulation miRNAs was conducted using custom-designed 384 well panels (Exiqon). Correlation analyses were performed using Spearman correlation in R (cor function). Missing values were neglected by only considering complete observations.

Out of 19 analyzed miRNAs, several were found to be highly correlated: miR-16-5p, miR-19a-3p, miR-19b-3p, and miR-93-5p were positively correlated (r = 0.71 to 0.96, see figure 1). In total, 12 miRNAs exhibited significant correlations to clinical parameters. Three miRNAs showed 5 or more significant correlations (see figure 2): miR-29b-3p was found to be positively correlated to P1NP (r=0.400, p=0.021), RANKL (r=0.421, p=0.021), CTX (0.415, p=0.013), TRAP5b by Trend (r=0.311, p=0.078), MAR (r=0.740, p<0.001), BFR/BS (r=0.536, p=0.022). miR-365a-3p was found to be positively correlated to P1NP (r=0.491, p=0.004), OC (r=0.368, p=0.030), CTX (r=0.337, p=0.048), TRAP5b (r=0.346, p=0.049), Tb.N (r=0.389, p=0.037). miR-550a-3p was found negatively correlated to BV/TV (r=-0.435, p=0.018) and Tb.Th (r=-0.429, p=0.020) and positively correlated to BS/BV (r=0.432, p=0.019), MAR (r=0.535, p=0.018). No correlations were found between age, OS, BS, ES, BS, Tb.Sp, MS, BS, Testosterone, OPG, BALp, iPTH, BMD L1-L4 and miRNAs

This is the first study reporting correlations between miRNAs, BTMs, histomorphometry and BMD. Serum levels of miR-365a-3p and miR-29b-3p, which are well-known modulators of bone formation in vitro, are positively associated to BTMs in patients with idiopathic osteoporosis. Mineral apposition rate which reflects new bone mineral being added to cancellous surfaces is strongly associated to miR-29b-3p and miR-550a-3p. Strong correlations were found between the miRNAs

	Pre-MP	Post-MP	MIO
<b>Bone Turnover Markers</b>			
iPTH (pg/mL)	34.9 ± 10.3	34.4 ± 13.5	26.3 ± 10.6
Serum CTX (ng/mL)	0.191 ± 0.12	0.323 ± 0.15	0.340 ± 0.17
TRAP5b (U/l)	1.9 ± 1.0	3.0 ± 0.8	2.8 ± 0.4
P1NP (µg/mL)	29.4 ± 22.2	42.0 ± 26.5	47.2 ± 22.3
BALp (µg/L)	16.1 ± 7.7	25.8 ± 12.7	22.3 ± 10.1
Osteocalcin (ng/mL)	13.7 ± 7.4	20.4 ± 11.3	19.4 ± 8.9
25 (OH) vitamin D (ng/mL)	32.7 ± 11.5	42.8 ± 26.3	30.2 ± 15.0
Osteoprotegerin (pmol/L)	2.3 ± 1.2	3.1 ± 1.9	2.5 ± 1.4
Free testosterone (nmol/L)	1.3 ± 0.9	1.0 ± 0.7	12.0 ± 4.9
<b>BMD Values</b>			
L1-L4 (T-score)	-2.3 ± 0.9	-2.4 ± 1.8	-2.1 ± 1.5
Total Hip (T-score)	-1.6 ± 1.2	-1.6 ± 1.2	-1.5 ± 1.1

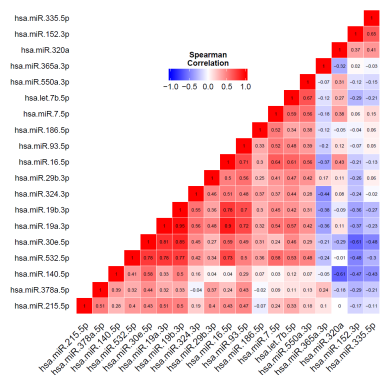


Figure 1. Spearman correlation matrix of 19 miRNAs with best discriminatory power between fracture patients and controls

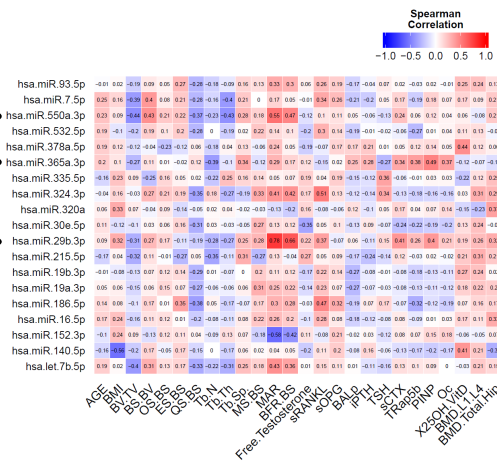


Figure 2. Heatmap depicting the Spearman correlation coefficients between bone turnover markers and histomorphometry and 19 selected miRNAs, which were previously shown to be significantly regulated in serum of low-trauma fracture patients and controls.