MiR-320a and miR-483-5p are over-expressed in osteoblasts from osteoporotic fractured hips

N. García-Giralt[1], L. De-Ugarte[1], S. Balcells[2], S. Ariño-Ballester[1], G. Yoskovitz[1], R. Güerri[1,3], L. Mellibovsky[1,3], R. Urreizti[2], X. Nogués[1,3], D. Grinberg[2], A. Díez-Pérez[1,3]

1. Musculoskeletal research group, IMIM (Institut Hospital del Mar d'Investigacions Mèdiques), Red Temática de Investigación Cooperativa en Envejecimiento y Fragilidad (RETICEF), Barcelona, Spain. 2 Departament de Genètica, Universitat de Barcelona, IBUB, Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), ISCIII, Barcelona, Spain. 3Servei de Medicina Interna, Hospital del Mar, Parc de Salut Mar, Universitat Autònoma de Barcelona (UAB), Barcelona, Spain

Introduction

MicroRNAs are important regulators of gene expression with documented roles in bone metabolism and osteoporosis. Moreover, the use of miRNAs constitutes a potential therapeutic approach. Our aim was to identify miRNAs differentially expressed in fractured compared to healthy bone. Additionally, we performed a miRNA profiling of primary osteoblasts to assess the origin of the differentially expressed miRNAs.

Methods

Total RNA was extracted from fresh femoral neck trabecular bone from women undergoing hip replacement due to either osteoporotic fracture (n=6) or osteoarthritis in the absence of osteoporosis (according to BMD measurements) (n=6), age and BMI matched, and from primary osteoblasts (at passage 0) obtained from knee replacement due to osteoarthritis (n=4). Samples were hybridized to the miRCURY LNA™ microRNA Array 7th (Exiqon, Denmark), in the manufacturers' facilities. QC tests, PCA plots and heat map hierarchical clustering were performed. For comparison of expression levels, the threshold was set at log fold change > 1.5 and a p-value < 0.05 (corrected for multiple-testing).

Results

Both PCA (Figure 1) and heat map (Figure 2) analyses showed that the samples clustered according to their biological group (fracture vs. non-fracture). However, one osteoporotic sample (O-500) appeared as outlier and was excluded.

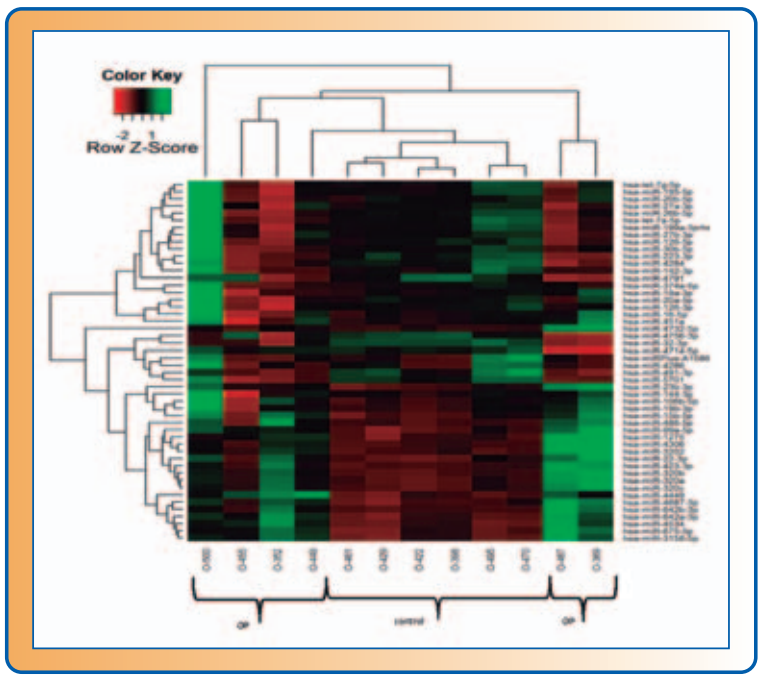

Figure 2: The heat map diagram shows the result of a two-way hierarchical clustering of microRNAs and samples. The clustering is done using the complete-linkage method together with the euclidean distance measure. Each row represents a microRNA and each column represents a sample. The microRNA clustering tree is shown on the left. The color scale illustrates the relative expression level of microRNAs. Red color represents an expression level below the reference channel, and green color represents expression higher than the reference. The clustering was performed on all samples, and on the top 50 microRNAs with highest standard deviation. A small subset of microRNAs has been excluded from the heat map. The normalized log ratio values have been used for the analysis.

A subset of 82 microRNAs was found to be significantly differentially expressed between osteoporotic and control samples. Upon validation of 10 miRNAs with the lowest p-values, and for which a validated assay was available, using the miRCURY LNATM Universal RT microRNA qPCR assay, two of them were confirmed: miR-320a and miR-483-5p (Table 1). They are both over-expressed in the osteoporotic samples (and expressed in primary osteoblasts).

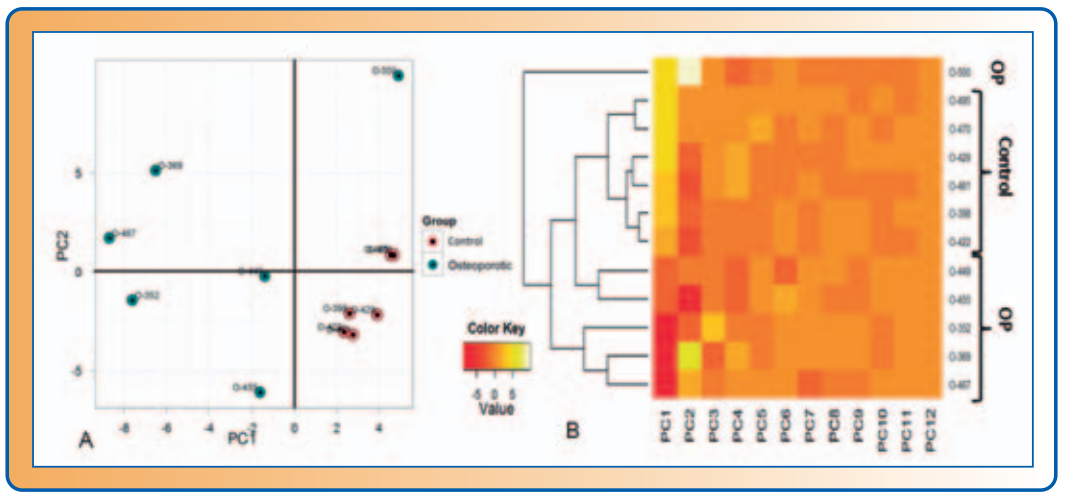

Figure 1: (A) Traditional and (B) Matrix PCA plot. The principal component analysis was performed on all samples, and on the top 50 microRNAs with the highest standard deviation. The normalized log ratio values have been used for the analysis. The features have been shifted to be zero centered, (i.e. the mean value across samples is shifted to 0) and scaled to have unit variance (i.e. the variance across samples is scaled to 1) before the analysis.

Overall, 790 and 315 different miRNAs were detected in fresh bone samples and in primary osteoblasts, respectively, 284 of which were shared (i.e. 35.8% of bone miRNAs were from osteoblasts) (Figure 3).

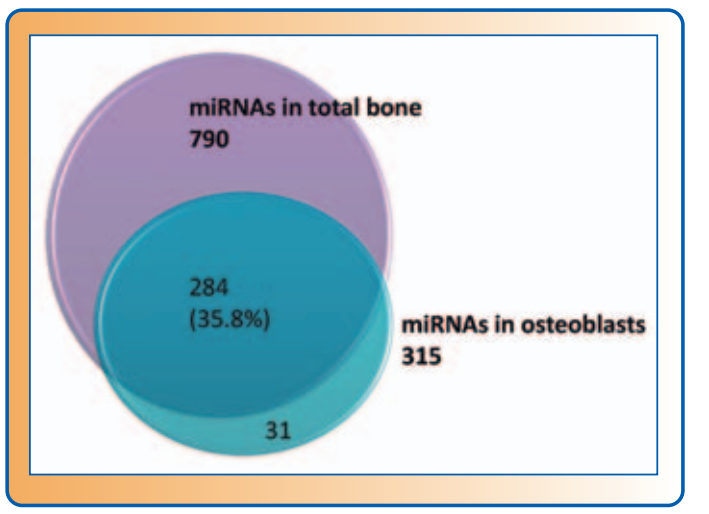

Figure 3: Venn diagram of total bone and osteoblast miRNA array.

Table 1: qPCR validated miRNAs which had reached significant values in the bone microRNA array

	average dCp		ddCp			
miR name	Control	OP	LogFC	SD	p	BH adj. p-value
hsa-miR-320a	2.08	5.42	-3.34	1.88	5.89E-05	5.30E-04
hsa-miR-483-5p	-4.48	-1.16	-3.32	1.98	1.59E-04	7.17E-04
hsa-miR-30c-1-3p	-7.25	-5.50	-1.75	1.28	4.62E-02	6.93E-02
hsa-miR-32-3p	-4.92	-3.76	-1.16	1.36	1.39E-01	6.93E-02
hsa-miR-142-3p	4.39	4.38	0.01	1.07	9.86E-01	6.93E-02
hsa-miR-155-5p	-0,66	-1,48	0,82	0,89	1,76E-01	6.93E-02
hsa-miR-223-3p	8.05	7.67	0.38	1.57	7.48E-01	8.26E-01
hsa-miR-542-5p	-3.34	-3.22	-0.12	0.73	8.26E-01	8.26E-01
hsa-miR-675-5p	-6.48	-4.87	-1.61	1.16	Not calculated	

Conclusions

Two osteoblast miRNAs, miR-320a and miR-483-5p, are over-expressed in osteoporotic fractures, which opens novel prospects for research and therapy.

There are no conflicts of interest