MiRNAs Involved in the Osteoblastic Function Are Altered in Human Osteoporotic Bone

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Introduction

It is well known that osteoblastic function is abnormal in osteoporotic bone. Moreover, we have observed by Real-time PCR that fresh human bone from fractured hips expresses low levels of osteoblastic markers (Alkaline phophatase, BMP2, osteocalcin and COL1A1). Our aim was to identify miRNAs differentially expressed in bone samples of fractured compared to healthy individuals. Additionally, we performed a miRNA profiling of primary osteoblasts (hOB) to assess the origin of the differentially expressed miRNAs.

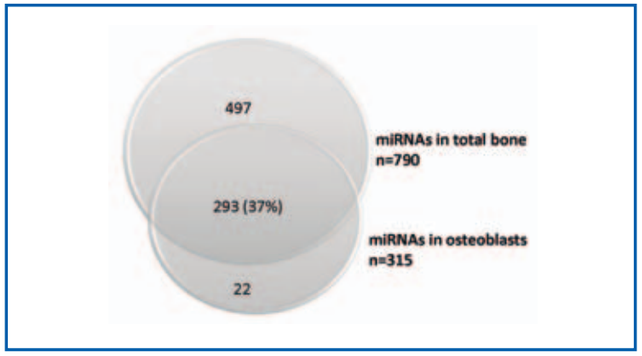
Methods

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 hOBs 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. For comparison of expression levels, the threshold was set at log fold change > 1.5 and a p-value < 0.05 (Benjamini and Hochberg corrected).

Results

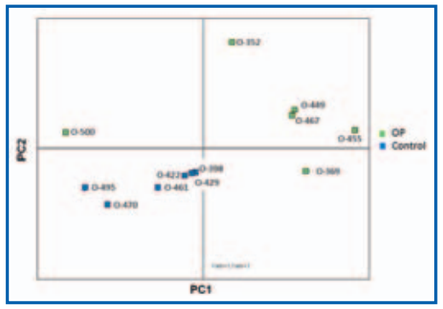
Overall, 790 miRNAs were detected in fresh bone samples, from which 293 (37%) were also detected in hOBs (Figure 1).

Figure 1: Venn diagram of total trabecular bone and osteoblast miRNA array



A subset of 82 microRNAs was found to be significantly differentially expressed between osteoporotic and control samples. Upon validation of 8 miRNAs with the lowest p-values, and for which a validated assay was available (miRCURY LNATM Universal RT microRNA qPCR assay), two of them were confirmed: miR-320a and miR-483-5p (Table 1). They were both found over-expressed in the osteoporotic samples (and expressed in hOBs).

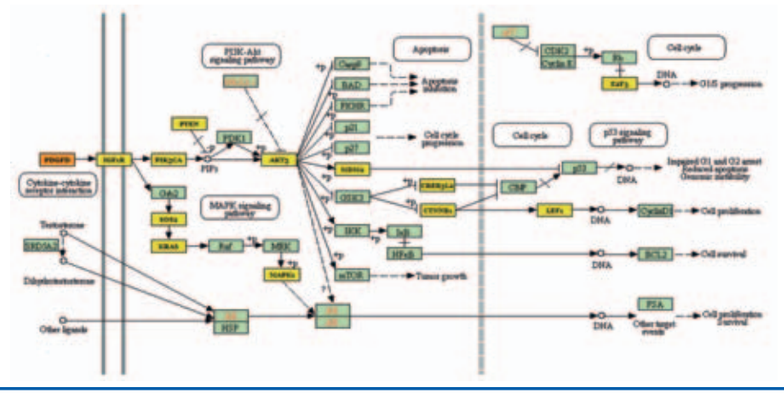
Figure 2: Principal component analysis was performed on all samples. Normalized (dCp) values were used for the analysis. Samples are clustered based on their biological group; however, sample O-500 appears to be an outlier.



The intersection pathways involving genes targeted by miR-320a and miR-483-5p are mainly prostate cancer (4.496403e-14), PI3K-Akt signalling (5.614388e-08) and focal adhesion (6.000918e-07) (Figure 3). It is noteworthy that these three pathways share many genes, such as: SOS2, IGF1R, PDK1, PDGFD, PIK3CA, AKT3, PTEN and MAPK1. Most of these genes are involved in cell proliferation and survival signalling, suggesting that a dysfunction in the osteoblastic cell renewal is occurring in the osteoporotic bone.

Figure 3: Pathways intersection of miR-320a and miR-483-5p using the DIANA-miRPath web server (1)

Prostate cancer pathway



PI3K-Akt signalling pathway

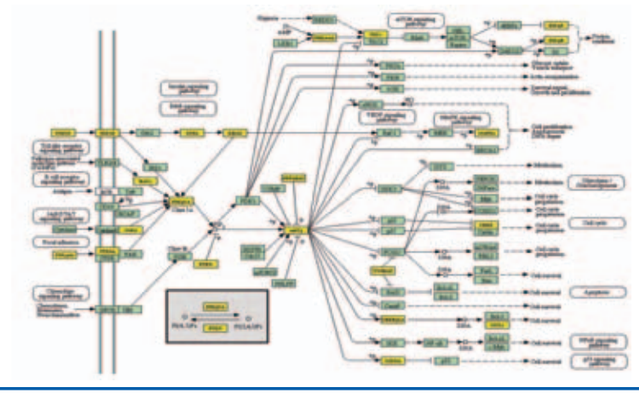


Table 1. qPCR-validated miRNAs that reached significant values in the microRNA array

miR name	average dCp Control (n=6)	ddCp OP (n=5)	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-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[1]

[1]miR-675-5p was detected in a low number of samples, which precluded statistical comparison between groups.

Conclusion

In conclusion, osteoporotic bone has an altered widespreadly osteoblast function at both key osteoblast gene expression as well as miRNAs expression levels.

References

(1) I. S. Vlachos, N. Kostoulas, T. Vergoulis, G. Georgakilas, M. Reczko, M. Maragkakis, M. D. Paraskevopoulou, K. Prionidis, T. Dalamagas, A. G. Hatzigeorgiou. DIANA miRPath v.2.0: investigating the combinatorial effect of microRNAs in pathways Nucleic Acids Research 2012 (Web server issue)