

Tumorigenic and Non-Tumorigenic Osteosarcoma Cell Subpopulations Exhibit Distinct miRNA Expression Profiles.

Elham Nasri MD., Padraic P. Levings PhD., Maria V. Guijarro PhD., Ali Zarezadeh MD., Margaret E. White, E.M. Viktoria Hyddmark, Yuan Lu, Glyn D. Palmer PhD., Steven C. Ghivizzani PhD., C. Parker Gibbs MD.
Musculoskeletal Oncology Laboratory, Department of Orthopaedics and Rehabilitation,
University of Florida, Gainesville, Florida

Background: Osteosarcoma (OS) is the most common primary malignant bone tumor in children and adolescents. Despite aggressive treatment around 40% of patients still die of their disease, which may be due to the heterogeneous nature of the tumor. Previously in our lab, we were able to identify and isolate tumorigenic and non-tumorigenic cells within the same tumor based on their ability to activate an exogenous Oct-4/GFP human promoter. Oct-4/GFP positive fraction probed to be at least 100-fold more tumorigenic than Oct-4/GFP negative fraction. The global transcription profile of these two populations revealed G2/M, spindle assembly checkpoint override and proliferation pathways more active in GFP positive cells. Stress induced differentiation and reversion pathways were more active in Oct-4/GFP negative cells.

There are several molecular mechanisms controlling the expression of genes. One important level of post-transcriptional gene expression regulation is microRNA. MicroRNAs (miRNA, miR) are non-coding small RNAs that regulate gene expression by targeting mRNAs. Accumulating evidence has shown that miRNAs are involved in multiple processes in cancer development and progression; however their role in intratumoral heterogeneity remains to be elucidated.

In this study we investigated the expression of 95 cancer-related miRNAs in tumorigenic and non-tumorigenic subpopulations isolated from the same tumor in two human osteosarcoma cell lines established from two patient biopsies (OS156, OS521). We believe these studies will help to identify novel targets for treatment, prevent the relapse of the disease and finally improve the patients' survival.

Objective: To establish miRNA profiles of tumorigenic and non-tumorigenic OS cell populations and identify putative mRNA targets.

Methods: Human OS cell lines, OS156 and OS521, were established from patient biopsies and transfected with the hOct4/GFP reporter. Subcutaneous tumors were generated in NOD/SCID mice by injection of Oct4/GFP+ cells isolated by Fluorescence Activating Cell Sorting (FACS). End point (1.5 cm) tumors were harvested and sorted by FACS into GFP positive and negative. Cell fractions from three different passages (each as one biological replicate) from each cell line were used to extract RNA and perform miRNA expression analysis using the Quantimир RT & PCR system (SBI System Biosciences, Mountain View, CA). For each cell line, results were analyzed by t-student test. TargetScan Human 6.2 and DIANNA were utilized for miRNA target prediction analysis.

Results: Analyzed data showed that 22 miRNAs in OS521 and 16 miRNAs in OS156 were upregulated significantly (p value <0.05) in the GFP positive fraction vs. GFP negative. Of these, a total of 12 miRNAs (miR-7, 20-a, 15b, 18-a, 25, 30a-3p, 106-a, 103, 93, 155, 221, 222) were common to both OS521 and OS156. A total of 4 miRNAs were found to be down-regulated in the GFP+ fraction relative to GFP- in OS156 (miR-134, miR-202, miR-206, miR-153) and one in OS521 (miR-153) (p value <0.05). Thus, only one miRNA, MiR-153, was significantly down-regulated in both cell lines.

To elucidate possible regulatory functions of the differentially expressed miRNAs, TargetScan and DIANNA prediction software were used to identify putative mRNA targets. Further validation was performed by microarray-based mRNA expression profiling to identify mRNA targets that demonstrated corresponding down-regulation within the same OS cell populations. In GFP+ cells, validated mRNA targets of the upregulated miRNAs (miR-15b, 18a, 20a, 93, 106a, 221) included G2-M (ATM, CHEK1 and WEE1) and G1-S checkpoint proteins (TP53, CDKN2A and CDKN2B). Down-regulation of these mRNAs suggests a loss of cell cycle control and deregulated cell proliferation which is consistent with the phenotype of tumorigenic GFP+ cells. Conversely, validated mRNA targets of miR-153 (down-regulated in the GFP+ fraction of both OS cell lines) suggest a possible tumor suppressor function for this miRNA. Among its targets are IRS2, which is known to enhance tumor growth via activation of MAPK signaling. Loss

of miR-153-mediated IRS-2 suppression in GFP+ cells is again consistent with their tumorigenic phenotype.

Conclusion: This is the first study to examine the expression of miRNAs in tumorigenic and non-tumorigenic subpopulations of OS. We have identified differentially expressed miRNAs associated with each subpopulation and their putative mRNA targets which can be explored further to elucidate the key regulatory mechanisms that drive tumorigenesis in OS.