Differential Mirna Expression In Osteosarcoma Tumor-initiating Cells And Their Reverted Progeny

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Disclosures:

Introduction: 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 contribute 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 enriched fraction probed to be at least 100-fold more tumorigenic than Oct-4/GFP depleted fraction. Gene expression profile revealed G2/M checkpoint override in Oct4-GFP positive cells and stress induced reversion/differentiation more in Oct-4/GFP negative cells.

One important level of gene expression regulation is miRNA. 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 OS and intratumoral heterogeneity remains to be elucidated. In this study we aimed to investigate the expression of 95 cancer-related miRNAs in tumorigenic and non-tumorigenic subpopulations isolated from the same tumor in two human osteosarcoma cell lines (OS156, OS521).

Methods: OS521Oct-4/GFP and OS156 Oct-4/GFP were generated by subcutaneous injection of Oct-4/GFP positive cells to NOD/SCID mice. Endpoint (1.5 cm) tumors harvested and sorted by FACS into GFP positive and negative fractions as described in (1). RNA extraction and RT-PCR were performed using Quantimir RT & PCR system (SBI System Biosciences, Mountain View, CA) respectively. Targetscan Human ver.6.2 and miRD were utilized for miRNA target prediction.

Results: A total of 24 miRNAs in OS521 and 16 miRNAs in OS156 were found downregulated in the GFP negative relative to the GFP positive population. 12 miRNAs were differentially downregulated in GFP negative fraction in both OS cell lines. Among them, miR-15b, 18a, 20a, 93, 106a seem to play a crucial role in controlling the cell cycle mainly by targeting the G2-M checkpoint (ATM, CHEK1, WEE1). MiR-221, 15b, 93 have been shown to regulate G1/S checkpoint by targeting TP53, CDKN2A and CDKN2B. 4 miRNAs (miR-206, 134, 202, 153) in OS156 and one miRNA in OS521 (miR-153) were found upregulated in GFP negative vs. positive fraction. MiR-153 was differentially upregulated in GFP negative subpopulation in both cell lines, and it has been shown to act as a tumor suppressor by specifically targeting important growth signaling mediators, like Irs-2.

Therefore differential expression of these miRNAs in the GFP negative fraction would result in activation of the cell cycle checkpoints and growth arrest, which correlates with our in vitro data. Our miRNA expression profile show clear differences among tumor initiating and non-tumor initiating cells mainly in proliferation but also in differentiation and survival under cellular stress, supporting previous data from our lab.

Significance: This is the first study to examine the expression of miRNAs in osteosarcoma tumor initiating cells and following their reversion in vivo. Our results suggest that miRNA expression may be a key regulatory mechanism behind intratumoral heterogeneity.


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