RNA-Sequencing Reveals Direct Targets of TumorSuppressor miR-203 in Human MammaryEpithelial Cells

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Repository Citation
Boardman, Alexander P.; Pedanou, Victoria E.; Simone, Tessa M.; and Green, Michael R., "RNA-Sequencing Reveals Direct Targets of Tumor Suppressor miR-203 in Human Mammary Epithelial Cells" (2017). University of Massachusetts Medical School. Senior Scholars Program. Paper 244.
http://escholarship.umassmed.edu/ssp/244

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Keywords
breast cancer, metastasis, anoikis, epithelial cells, miR-203

Comments
Alexander P. Boardman participated in this study as a medical student as part of the Senior Scholars research program at the University of Massachusetts Medical School. This poster was presented on Senior Scholars Program Poster Presentation Day at the University of Massachusetts Medical School, Worcester, MA, on May 3, 2017.

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RNA-Sequencing Reveals Direct Targets of Tumor Suppressor miR-203 in Human Mammary Epithelial Cells

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Background: Breast cancer is the leading cause of cancer-related mortality in women worldwide. Since a significant portion of cases present with or progress to metastatic disease, furthering our understanding of metastasis is critical to develop better treatments. Epithelial cells maintain contact with the extracellular matrix (ECM) predominantly via integrin engagement, a process required for tissue integrity and barrier function. In non-transformed cells, loss of ECM adhesion promotes a specialized form of programmed cell death, anoikis. In order for efficient metastasis to occur, breast tumor cells must evade anoikis. miR-203, known to be down-regulated in several cancers, was found by our lab to be induced ten-fold 24 hours following detachment in breast epithelial cells, but not invasive triple negative breast cancer (TNBC) cells, suggesting that miR-203 may participate in promoting anoikis. Interestingly, more invasive breast cancer cell lines have been shown to express miR-203 at significantly lower levels than those of less invasive lines.

Objectives: Since restoration of miR-203 expression ectopically is not feasible in a clinical setting, we sought to identify and characterize miR-203 target genes in order to provide a pharmaceutical platform for restoration of anoikis sensitivity in metastatic breast cancer.

Methods: We performed traditional RNA-sequencing (RNA-Seq) coupled with immunoprecipitation of the RNA-induced silencing complex (RISC; Ago2 RIP-Seq) in MCF-10A, an immortalized, but non-transformed breast epithelial cell line, overexpressing precursor miR-203 or an empty vector control. MDA-MB-231, triple negative ductal carcinoma cells, were used as our invasive comparison cell line.

Results: Here we show that miR-203 induction in detached MCF-10A cells is due to loss of integrin signaling. Our coupled RNA-Seq and Ago2 RIP-Seq approach revealed 72 potential candidates, 42 of which were predicted miR-203 targets based on the TargetScan algorithm. We subjected the candidates to stringent characterization and found 9 bona-fide miR-203 targets that promote cell death when inhibited. Among these, WDR69, PRKAB1, PRPS2, and HBEGF were significantly elevated in TNBC tumor samples, as determined by RNA-Seq analysis in The Cancer Genome Atlas (TCGA).
**Conclusion**: Understanding the mechanisms by which cells evade anoikis during tumor dissemination is crucial to developing more effective therapies in breast cancer. miR-203, which is expressed at very low levels in more invasive breast cancers, is a positive regulator of anoikis that is upregulated in response to loss of contact with the ECM. Our combined RNA-sequencing screen revealed 42 direct miR-203 targets. Inhibition of 9 bona-fide targets promoted cell death, suggesting that they are negative regulators of anoikis. WDR69, PRKAB1, PRPS2, and HBEGF were all significantly elevated in TNBC tumor samples relative to less invasive samples, likely a consequence of low miR-203 expression. The identified genes represent potential pharmaceutical targets for novel breast cancer therapies.