Case Study
HTG EdgeSeq
miRNA Whole Transcriptome Assay

HTG EdgeSeq miRNA Whole Transcriptome Assay can help reveal miRNA regulation of bone homeostasis and remodeling

Problem: Studying miRNAs in osteoclasts is challenging

Osteoclasts (OCs) are specialized cells that help break down bone tissue for repair and remodeling. Osteoclasts develop from myeloid precursor cells in a process that is largely driven by two proteins, RANKL and M-CSF[1]. Recent studies have demonstrated the role of miRNAs as both positive and negative regulators of OC generation, survival, as well as OC function in bone remodeling[1-4] *(Figure 1)*.

MicroRNAs (miRNAs) are short non-coding regulatory RNAs. They are post-transcriptional modulators of gene expression and have important roles in many varied cellular processes ranging from cell proliferation, differentiation, migration, and survival. miRNAs have also been shown to modulate bone homeostasis and impact OC bone remodeling in osteoporosis[5-9], making miRNAs potential therapeutic targets for bone-related disorders[10].

Studying miRNAs in primary OCs is challenging, because the localization of OCs within bone cavities makes extraction difficult. Obtained samples of OC cells are often only partially pure and may contain a mixed population of cells in different developmental states[1]. For this reason, researchers have begun looking to other tissues outside of bone to study OC biology.

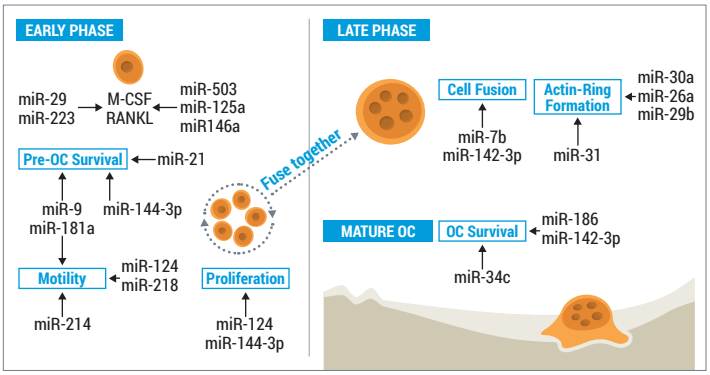
Solution: Measuring circulating miRNAs

In their 2019 paper in Frontiers in Immunology, Lozano *et al.,* provide a detailed view of technologies focused on the detection and quantification of miRNAs. The authors note that while many current technologies used for miRNA profiling such as RNASeq, qPCR, hybrid-capture, and NGS have enabled more sensitive detection of miRNAs, they can show a significant amount of bias in the data due to heavy amounts of pre- and post-processing. For instance, the quality, quantity, and composition of the RNA sample can vary significantly between studies depending on the RNA extraction, isolation, and amplification methods used for sample preparation. These additional steps can introduce unwanted bias.

The authors noted that some methods, such as the HTG EdgeSeq miRNA Whole Transcriptome Assay, provide miRNA measurement without RNA extraction. The authors go on to point out that this technology allows for the direct measurement of miRNAs from as little as 15 µl of plasma or serum fluid. The lack of extraction or cDNA synthesis avoids the bias typically associated with extracted samples and increases the sensitivity and accuracy of measurements as detected by a gradient of miRNA measurements.

The authors did note that cross reactivity is higher compared to qPCR assays, however, the HTG EdgeSeq Whole Transcriptome Assay results in data that is easy to analyze, and shows excellent correlation to RNASeq (>0.95) when used on tissue samples[13] as well as quantitative PCR (qPCR,0.93) and digital PCR (dPCR, 0.94) when tested with plasma samples[14]. The HTG EdgeSeq Assay results were also closest to the RNASeq results for FFPE samples, with a >95% concordance.

Figure 1. *Graphic depicting miRNA regulation of osteoclast differentiation. Arrows and bars indicate positive and negative regulation by miRNA, respectively. Adapted from Lozano et al.*[1]



Significance: The HTG EdgeSeq miRNA Whole Transcriptome Assay reduces handling bias and processing time, while simplifying identification of miRNAs

Researchers at the University of Montpellier identified the HTG EdgeSeq miRNA Whole Transcriptome Assay as a promising tool for studying the role of miRNAs in osteoclast differentiation[1]. The HTG EdgeSeq miRNA Whole Transcriptome Assay can simultaneously measure 2,083 miRNAs without the need for RNA extraction. Simultaneous measurement of nearly the entire known library of human miRNAs allows for more consistent and comparable data than qPCR with reduced bias. Skipping the RNA extraction step eliminates a major source of bias and also means there are fewer steps for faster, easier processing and higher yields than traditional RNAseq workflows. Lastly, the HTG EdgeSeq miRNA Whole Transcriptome Assay also requires low input volumes, making it a powerful tool for miRNA profiling in difficult to obtain tissue like osteoclasts.

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