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Optical Sensing of miRNA Activity in Cells

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We have developed a technology for the profiling of miRNA expression in intact cells. The approach is based on sensor oligonucleotides, which upon entering the cell, bind specific miRNA targets, are cleaved as a result of this binding, and produce a fluorescent signal that is proportional to the abundance of the miRNA target.

Specifically, the sensor oligonucleotides are completely complementary to a target miRNA species, are non-stabilized around the seed region (the region cleaved by the miRNA-RISC), and are labeled with a fluorescent dye and a quencher at their 5'- and 3'- end respectively. Upon entering the cell, these oligonucleotides engage the target miRNA by complementary base pairing. This leads to recruitment of the RNA induced silencing complex (RISC) to the duplex. The complex cleaves the sensor oligonucleotide and the miRNA is free to "catalyze" subsequent clevage reactions. The cleavage of the sensor oligo leads to separation between the dye and the quencher, and a resultant fluorescent enhancement that can be measured.

We have demonstrated the feasibility of this method for the sensing of the pro-metastatic miRNA-10b in cell-free extracts and intact cells using human and murine breast adenocarcinoma cell lines.

The miRNA epigenome represents a fundamental molecular regulator of metastasis. Consequently, developing tools to understand metastatic changes at the miRNA level can lead to the mapping out of a comprehensive and systematic atlas of cancer progression. The described technology is potentially transformative because it addresses this important issue. Furthermore, the technology has broad implications and can be utilized in any model system or clinical scenario to answer questions related to microRNA function. Specifically, the technology can help distinguish, assess, and/or monitor cancer stages and progression; aid the elucidation of basic mechanisms underlying cancer initiation and progression; facilitate early cancer detection and/or cancer risk assessment; and facilitate/accelerate the process of drug discovery.