Cellular context in epigenetics: Quantitative multicolor imaging and automated per-cell analysis of miRNAs and their putative targets

James R. Mansfield, 1 Gerard Nuovo, 2 Matt Coffey, 3 Mitch Phelps, 2 David Cohn 2

1 Caliper Life Sciences, Hopkinton, MA USA; 2 Ohio State University, Columbus, OH; 3 Oncolytics, Calgary, AB

Abstract

Epigenetics in general and micro RNA (miRNA) in particular are an important and growing field of research, and while significant advances in the role of miRNA in a variety of diseases including cancer have been made, the majority of the information on the relationship between miRNA and its putative target proteins have been made on homogenized tissue, which, while useful, provides no information on tissue- or cell-specific signatures or relationships. Developments in spectral imaging have made it possible to image and quantitate samples labeled for both a miR and its putative target, using either brightfield or fluorescence labeling. Advances in automated morphometric and cellular quantitation enable the extraction of quantitative, tissue-specific measures of marker in each cell of a tissue section. This per-cell quantitative data can then be displayed as scatter plots, in a manner analogous to flow cytometry data, and multimarker phenotypes can be determined from threshold-based quadrant analysis. Microscopy-based multi-analyte methods offer the benefit of visualizing miRNAs and their putative targets within the context of disease-specific molecular anatomy and on a per-cell basis.

This study describes the means by which tissue sections labeled for multiple markers (proteins and microRNAs) can be analyzed and then be displayed as scatter plots, in a manner analogous to flow cytometry data, and multimarker phenotypes can be determined from threshold-based quadrant analysis.

Two products have been specifically designed for this purpose: the Nuance™ multispectral imaging system, which combines a sophisticated liquid-crystal tunable filter (LCTF) approach to spectral imaging and which fits easily on any microscope with a computer port, and the InForm™ imaging analysis software package, which enables the user to train it to differentiate complex tissue types and then extracts per-cell quantitative data. Combined with multicolor, multimarker staining of tissues, these products can easily deliver quantitative per-cell data on the amount of each marker in every individual cell in each tissue type. To show their utility, these systems have been applied to a number of ISH and IHC stained tissues, including two samples dual-labeled for a miR and its putative target protein. In all cases, the Nuance imaging system combined with InForm software analyses increased contrast and legibility in multicolor samples (both brightfield and fluorescence), increased the accuracy of the quantitation of each marker in the samples, and provided the per-cell quantitative data needed to explore the relationship between miR and putative target protein on the tissue and cellular level in complex tissue sections.

Per-cell quantitation of miRNA and target protein

Combined ISH and IHC staining and quantitation

miR and protein coexpression assay

Multispectral imaging technology

Morphologic and cellular segmentation

inForm™ breast cancer ER PR coexpression assay

Nuance™ Multispectral Imaging Systems

Unmixing of Overlapping Chromogens

Once unmixied, stains can be measured accurately.