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MICRORNA DETECTION IN TUMOR TISSUE BY *IN SITU* HYBRIDIZATION

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Abstract: *In this paper the Authors describe the main functions of microRNAs (miRNAs) along with well acknowledged methods of their detection in biological materials. They include serum, plasma, urine, normal human samples and neoplastic tissues. The Authors also discusses about the relevance of bright field in situ hybridization (ISH) methods for miRNA detection in revealing the cells of origin of specific miRNAs. In particular, the paper highlights the automated ISH protocols for miRNA detection which have recently been proposed. These techniques will enable investigators to further explore the biological role of miRNAs.*

microRNAs (MIRNAS)

miRNAs are small (18-25 nucleotides) non-coding RNAs that modulate gene expression by binding to complementary sequences on target messenger RNA transcripts.

miRNAs FUNCTIONS

miRNAs regulate protein expression by suppressing mRNA translation and/ or promoting mRNA degradation. The importance of miRNA functions in many physiologic processes and pathologic conditions is confirmed by the fact that more than 30% of mRNAs are regulated by one or more miRNAs[1-3]. Given their role in regulating protein expression, and therefore homeostasis and epigenetics, MiRNAs have been a topic of research interest. Their potential utility as predictive and prognostic biomarkers has been suggested[4-11].

METHODS OF miRNA MEASUREMENT AND DETECTION

Measurements of miRNA levels in serum, plasma and tissue extracts using qRT-PCR oligonucleotide microarray, or miRNA-sequencing have been reported[4,5,8] (Table 1). However, these techniques are not able to determine the cellular origin of miRNAs in tissues. For a precise analysis of the topographical expression of miRNAs in tissues and therefore an in-depth understanding of miRNA function in development, diseases, and tumors it is crucial to employ *in situ* hybridization (ISH). For ISH is mandatory to optimize tissue morphology and preservation for a better detection of its localization. Published ISH protocols were developed using frozen tissues[12], in which the morphology is not as detailed as in formalin fixed, paraffin embedded (FFPE) tissues. Recent advances in ISH have enabled detection of miRNAs in FFPE tissues using locked nucleic acid (LNA) probes. The

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TABLE 1. METHODS OF MIRNA MEASUREMENT AND DETECTION FROM THE LITERATURE.

Material(s)	Method(s)
Serum, plasma, urine	qRT-PCR, sequencing
Tissue	qRT-PCR, sequencing
Tissue (frozen)	ISH
Tissue (FFPE)	manual ISH (FISH, bright field)
	semi-automated ISH
	automated ISH

FFPE, formalin fixed, paraffin embedded; ISH, *In situ* hybridization; FISH, fluorescent *in situ* hybridization.

ability to perform ISH on FFPE tissues is critical, also because this increases sample availability: archived tissues can be examined retrospectively.

CONVENTIONAL ISH METHODS

Fluorescent ISH allows to detect weak signals: the operator, indeed, can enhance the laser power promotes the signal brilliance. However, the signal detection requires a fluorescent microscope and the hybridized samples must be conserved at 4°C, avoiding the light exposure. Importantly, in dark field microscopy, the morphology and the pathological environment is not so clear as in bright field microscopy. Manual ISH is a procedure that brings variability among samples, and limits the use of ISH.

SEMI-AUTOMATED AND AUTOMATED ISH METHODS

To overcome these criticalness a bright field miRNA ISH using a semi-automated method has been set up (manuscript submitted for publication). The workflow of the protocol is subdivided in two parts: the first part (tissue preparation, permeabilization, hybridization) is manual, while the second one (signal detection) is automated employing an automated immunostainer. The manual part takes about a half day and the automated procedure takes about three hours. This method provides a good morphological detail and optimal signal localization (Figure 1).

Interestingly, a reliable and high throughput ISH method and optimized tissue fixation conditions for FFPE tissues has been recently developed[13]. This ISH method was validated by visualizing four miRNAs using LNA probes and the Discovery Ultra Ventana™ platform[13].

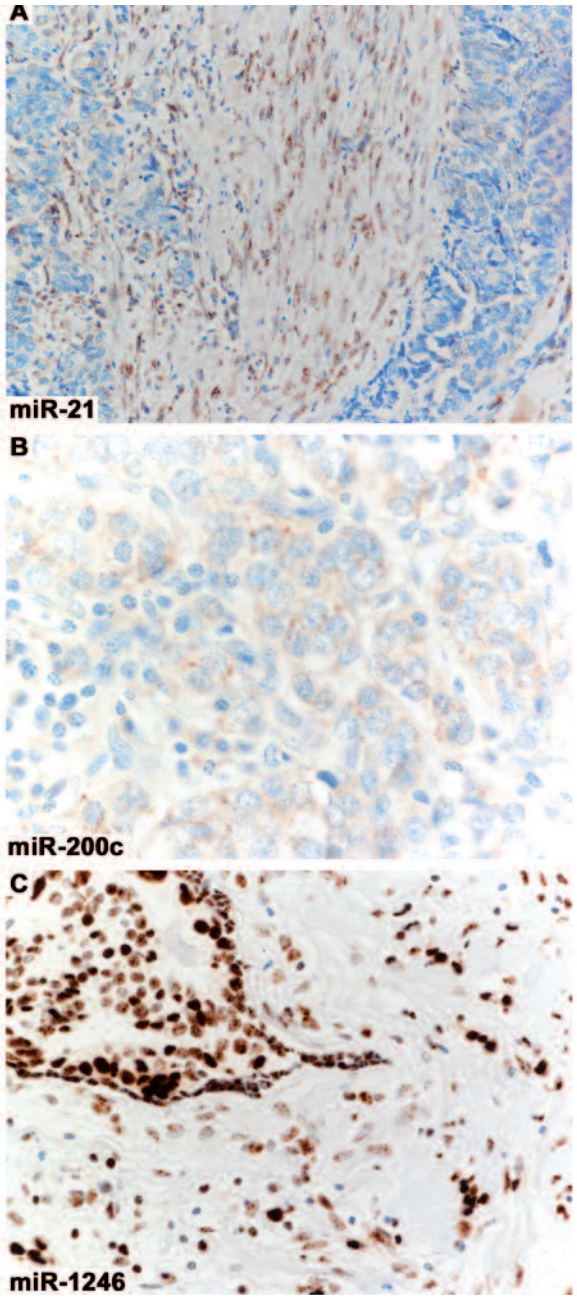

Figure 1. Examples of microRNAs (miRNAs) detection by *in situ* hybridization (ISH) in formalin fixed, paraffin embedded tissue sections. **A**, Specific brown miRNA-21 ISH signal using DAB substrate is clearly observed in the stromal compartment of an ovary carcinoma, whereas is less recognizable in the neoplastic cells. **B**, miRNA-200c ISH signal is cytoplasmic in neoplastic cells of breast carcinoma. **C**, miRNA-1246 ISH signal is observed in the nucleus of breast carcinoma cells. Semi-automated ISH method, Hematoxylin counterstain. Magnification: x200 (**A**), x400 (**B, C**).

CONCLUSIONS

In-depth understanding of miRNA functions involves detailed expression patterns of miRNAs in tissues. Consistent ISH methods can also be a useful tool to investigate the utility of miRNAs as

clinical biomarkers. Novel and semi-automated ISH methods will enable investigators to explore further the biological role of miRNAs and miRNA-associated gene regulatory networks in development, disease conditions and tumors.

Conflict of Interests:
The Authors declare that they have no conflict of interests.

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