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 miRNAs⁴⁻⁵. 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 suggested⁶⁻¹¹.

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 reported⁴,⁵,⁸ (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 tissues¹², 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
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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**REFERENCES**