Editorial

Identification of optimal reference RNAs to normalize miRNA expression data by qRT-PCR in formalin-fixed, paraffin-embedded lymph node tissue

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We recently read the interesting paper of Inada *et al.*, published on *Sci Rep* 2018 (1), regarding the identification of optimal reference RNAs for miRNA quantification using qRT-PCR in formalin-fixed paraffin-embedded (FFPE) lymph node tissues from patients with metastatic cancer. FFPE material represents an extensive repository of tissue samples with a long-term clinical follow-up, providing a valuable resource for translational research. Even though RNA degradation occurs, due to the formalin fixation process, it is feasible to extract miRNA from FFPE tissue and to perform qRT-PCR-based expression profiling. However, a crucial point in approaching accurate miRNA expression quantification using qRT-PCR is the choice of the most stably expressed endogenous references that should be used for data normalization. Actually, most of the investigations in the literature report arbitrarily chosen endogenous controls, including miRNA, snRNA and snoRNA, without any experimental validation of their stability or they commonly use U6 snRNA, although a growing body of evidence demonstrate its high expression instability across tissues (2). Moreover, according to the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines (3), normalization against a single reference gene is not acceptable and a combination of different reference genes should be used in order to produce reliable quantification. The paper of Inada *et al.* found a combination of three miRNAs as suitable endogenous references for miRNA quantification in FFPE lymph nodes. The miRNA combination, including miR-24, miR-103a and let-7a, has been obtained starting from 71 candidate miRNAs using four different statistical tools, preceded by a global mean normalization strategy. Those three miRNAs have been already reported with stable expression in tumor tissues, thus confirming authors' findings. We believe that Inada *et al.*'s article reflects a well-conducted study, in compliance with MIQE guidelines for accurate miRNA quantification using qRT-PCR. Moreover, one of the strength of the experimental method is the application of the Exiqon technology to miRNA quantification, ensuring the maximum sensitivity, specificity and accuracy of qRT-PCR system, as recently reported (4). Although the relatively small sample size may be considered a limitation of the study, we believe that Inada and colleagues' article could be considered a valuable starting point for other researchers who need to reliably quantify miRNA expression through qRT-PCR in FFPE lymph node tissues.

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