

Comparison of microRNA expression using different preservation methods of matched psoriatic skin samples

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Background

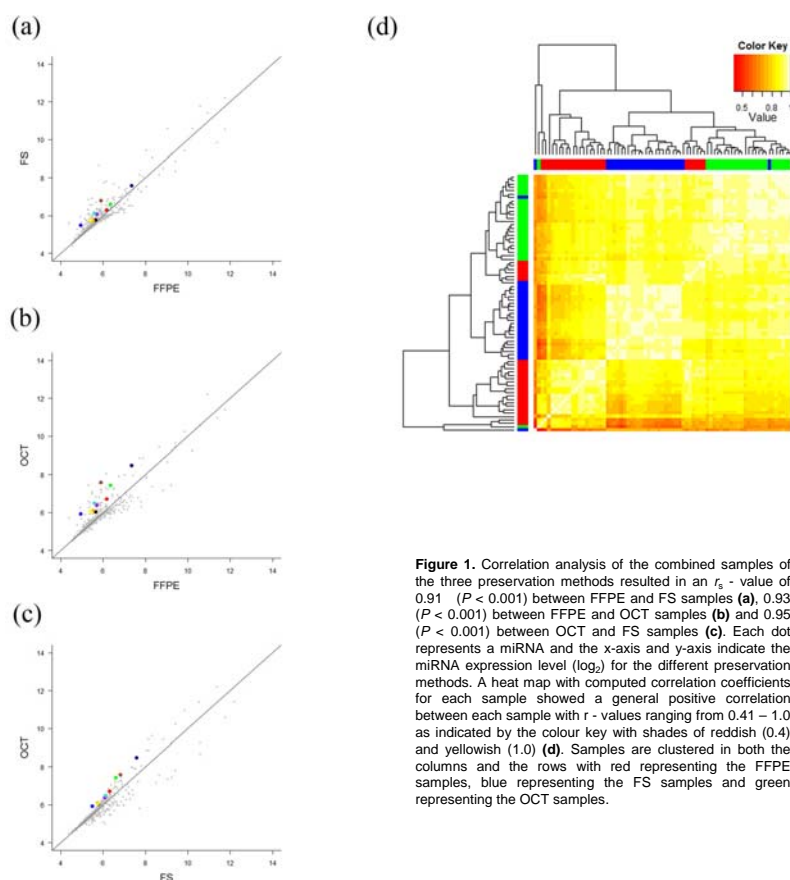
MicroRNAs (miRNAs) are small non-coding RNA molecules that modulate gene expression at the post-transcriptional level. The inflammatory skin disease psoriasis is characterized by a specific miRNA expression-profile that differs from normal skin. Extracting high quality RNA from human skin can be a challenge since the skin contains high levels of RNases. Furthermore, fixation of tissue samples using for instance formalin-fixation, paraffin-embedding (FFPE) causes extensive damage to the nucleic acids stored within the tissue, making subsequent RNA extraction and quantification challenging. Due to their small size (19-23 nucleotides) and lack of a poly A tail, miRNAs may be less affected by RNA degradation and damage than messenger RNAs (mRNAs).

Aim

To investigate the effect of three different preservation methods, FFPE, frozen (FS), and Tissue-Tek-embedding (OCT) on the global miRNA expression levels in matched lesional skin samples from 25 patients with psoriasis using the miRNA analysis platform miRCURY LNA™ MicroRNA array (v. 11.0) (Exiqon, Vedbaek, Denmark) and quantitative RT-PCR.

Result I

We found a strong correlation of the miRNA expression levels between the three different preservation methods of psoriatic skin samples with correlation coefficients (r_s - value, Spearman) ranging from 0.91 to 0.95 ($P < 0.001$) (Fig. 1a-c). Furthermore, a sample correlation matrix presented as a heat map with unsupervised hierarchical clustering showed an overall good similarity between each sample with correlation coefficients ranging from 0.41 – 1.0 as indicated by the colour key (Fig. 1d).



Result II

Using multi- and single-plex quantitative RT-PCR we confirmed the observations reported in the miRNA microarray (Fig. 2a-b, Fig. 3).

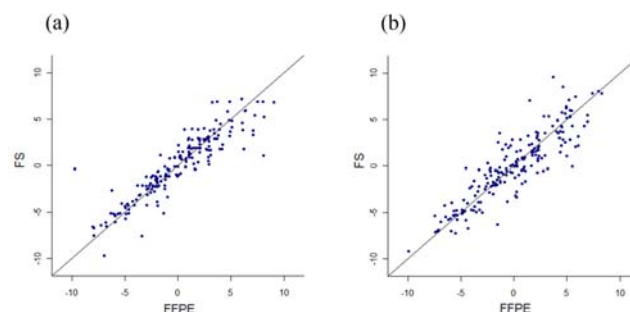


Figure 2. TaqMan® Array MicroRNA Cards (Applied Biosystems) (a) and MicroRNA Ready-to-Use Human panels (Exiqon) (b) were used to determine the miRNA expression level in a FFPE and FS sample from one patient with psoriasis. Correlation analysis between FFPE and FS resulted in an r_s - value of 0.92 ($P < 0.001$) (a) and 0.89 ($P < 0.001$) (b), respectively. Each dot represents a miRNA, the x-axis represents delta Ct - values from the FFPE sample and the y-axis represents the delta Ct - values from the FS sample.

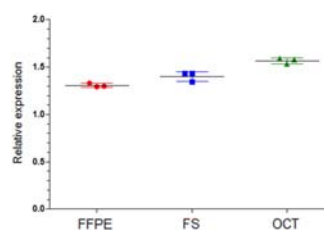


Figure 3. Single-plex qRT-PCR of isolated RNA from three patients with psoriasis was used to validate the expression of miR-21. No significant difference between the three preservation methods was found ($P > 0.05$). Individual samples were measured in triplicates. Data points for each preservation method including mean \pm standard deviations are shown. Data are expressed at the y-axis as relative units compared to RNU6B.

Conclusion and future perspective

We found a strong correlation of the miRNA expression levels between the three different preservation methods (FFPE, FS and OCT) of psoriatic skin samples with correlation coefficients ranging from 0.91 to 0.95 ($P < 0.001$). These observations were further confirmed with multi- and single-plex quantitative RT-PCR.

Our results demonstrate that miRNA detection in human skin is robust and reproducible irrespective of the preservation method used and thus, the miRNAs offer an appropriate and flexible approach in clinical practices and may hold great promise for biomarker and novel target discovery for skin diseases in the future.



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