Supporting Information

Analysis of circulating microRNAs and their post-transcriptional modifications in cancer serum by on-line solid-phase extraction capillary electrophoresis-mass spectrometry

Roger Pero-Gascon[1], Victoria Sanz-Nebot[*1], Maxim V. Berezovski[2], Fernando Benavente[1]

[1] Department of Chemical Engineering and Analytical Chemistry, Institute for Research on Nutrition and Food Safety (INSA·UB), University of Barcelona, Barcelona 08028, Spain

[2] Department of Chemistry and Biomolecular Sciences, University of Ottawa, Ottawa, Ontario K1N 6N5, Canada

[*]Corresponding author: vsanz@ub.edu (V. Sanz-Nebot, PhD)

Tel: (+34) 934021283, Fax: (+34) 934021233

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Optimized MS parameters

The MS parameters, optimized by infusion experiments with a 5000 $nmol \cdot L^{-1}$ let-7g-5p standard solution, were the following: capillary voltage 3500 V, drying gas temperature 350ºC, drying gas flow rate 6 $L \cdot min^{-1}$, nebulizer gas 7 psig, fragmentor voltage 225 V, skimmer voltage 70 V, OCT 1 RF Vpp voltage 300 V. Data were collected in profile at 1 spectrum/s between 100 and 3,200 *m/z*, with the mass range set to high resolution mode (4 GHz).

Serum samples extraction

According to the manufacturer's instructions, 0.3 mL of serum was mixed with 0.3 mL of PCA solution, centrifuged at 12,000 g and the aqueous phase (upper) was transferred to a new tube. To evaluate the extraction methodology, healthy control serum samples were spiked with 50 $nmol \cdot L^{-1}$ of the standard miRNAs, immediately after the first extraction to avoid degradation by endogenous serum RNases. Extraction with PCA was repeated twice more, until no protein was visible in the interface. Then, the aqueous phase was extracted with 0.2 mL chloroform to remove any residual phenol. Alternatively, for the extraction with the TRIzol Reagent, 0.3 mL serum was mixed with 3 mL of the TRIzol Reagent and 0.6 mL chloroform. In accordance to the manufacturer's instructions, the extraction was repeated only once. In both cases, drop dialysis of the obtained aqueous phases (upper) was performed with MF-Millipore membrane filters of mixed cellulose esters, with average pore size diameter of 25 nm and 25 mm of diameter (Millipore, Molsheim, France). The filter was floated with the glossy side up on a beaker with 50 mL of water. After 5 min for allowing the floating filter to wet completely, 100 µL of the sample extract was carefully placed on the center of the membrane and dialyzed for 1 hour at room temperature.

Quality parameters

All quality parameters were calculated from data obtained by measuring peak area and migration time (t_m) from the extracted ion electropherogram (EIE) considering the *m/z* of the most abundant miR-21-5p and let-7g-5p ions from the cluster resolved for the $[M-5H]^{5-}$, and the sodium and potassium adducts $[M-6H+Na]^{5-}$ and $[M-6H+K]^{5-}$.

An estimation of the LODs was obtained by analyzing low-concentration standard mixtures (close to the LOD level, as determined from the approach based on S/N=3). LOQ was determined from the approach based on S/N=10. Reproducibility in SPE-CE-MS was evaluated as the relative standard deviation (percent RSD) of peak areas and t_m. Linearity range was studied between 10 and 150 $nmol \cdot L^{-1}$. The lifetimes of the microcartridges was investigated by iteratively analyzing a standard mixture of 50 $nmol \cdot L^{-1}$.

Table S-1. Characteristics and molecular masses of the **(A)** standard miRNAs, **(B)** miRNA tentatively identified by SPE-CE-MS in a CLL-patient serum sample.

miRNA ID	Sequence	Modification	m/z $[M+2-5H]^{5-}$ a)		Calculated M_r ([M+2]) a)		
			Theoretical	Observed	Theoretical	Observed	Error (ppm)
(A) Standard miRNAs							
hsa-miR-21-5p (miR-21-5p)	UAGCUUAUCAGACUGAUGUUGA	5' phos	1415.5707	1415.5592	7082.89	7082.84	8
hsa-let-7g-5p (let-7g-5p)	UGAGGUAGUAGUUUGUACAGUU	5' phos	1426.9677	1427.9537	7139.88	7139.81	10
(B) miRNA tentatively identified in a CLL-patient serum sample							
hsa-miR-21-5p (miR-21-5p)	UAGCUUAUCAGACUGAUGUUGA	5' phos	1415.5707	1415.5485	7082.89	7082.78	15
hsa-iso-miR16-5p	UAGCAGCACGUAAAUAUUGGCGU	5' phos, 3'-U	1488.9890	1488.9723	7449.98	7449.90	11

a) M+2 corresponds to the most abundant ion of the isotopic distribution cluster resolved for the -5 molecular ion.

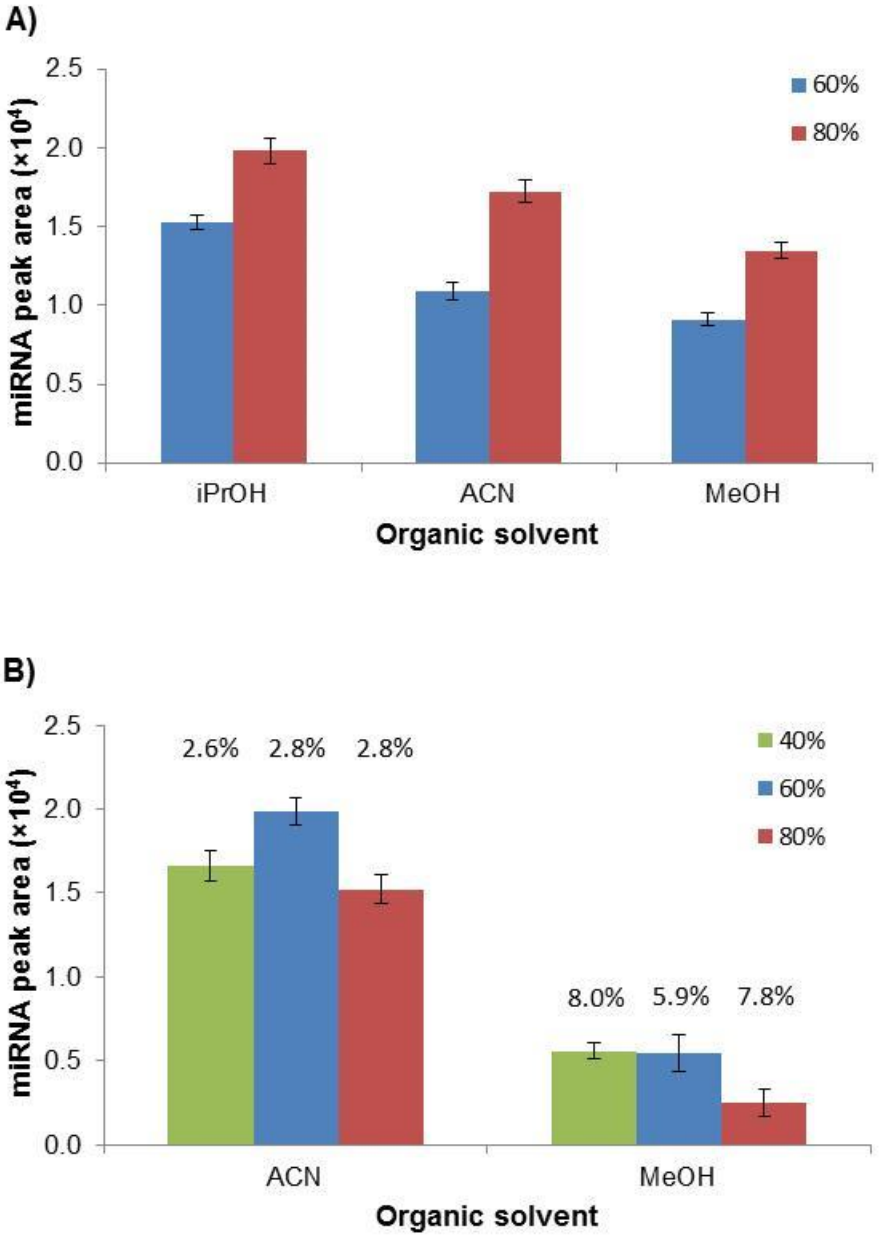

Figure S-1. Effect of the organic solvent **(A)** in the sheath liquid (CE-MS, 5,000 nmol·L^{-1} miR-21-5p standard) and **(B)** in the eluent (SPE-CE-MS, 50 nmol·L^{-1} miR-21-5p standard). Peak area of the injected (CE-MS) or eluted miRNA (SPE-CE-MS). All measurements were performed in triplicate (standard deviation is given as error bars, percentage RSD for migration times is given in numbers).

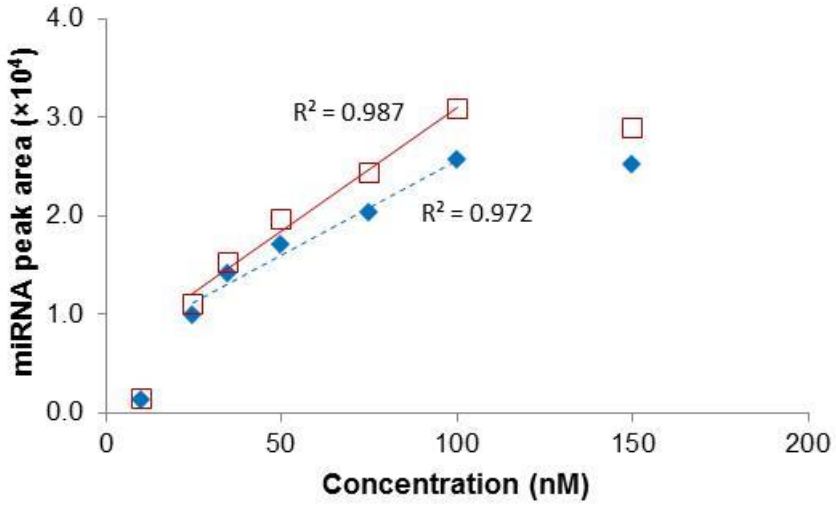

Figure S-2. Plot of peak area of the eluted miR-21-5p (♦) and let-7g-5p (□) vs concentration of the loaded standard mixture (930 mbar, 5 min). Regression line and R^2 value in the linearity range.

Table S-2. miRNAs reported in human B-cell chronic lymphocytic leukemia (CLL) plasma and cell samples used for the screening of endogenous miRNA in a CLL-patient serum sample by SPE-CE-MS (miRBase 21, http://www.mirbase.org [49]).

miRNA ID	Sequence	Length, nt
hsa-miR-15a-5p	UAGCAGCACAUAAUGGUUUGUG	22
hsa-miR-16-5p	UAGCAGCACGUAAAUAUUGGCG	22
hsa-miR-16-2-3p	CCAAUAUUACUGUGCUGCUUUA	22
hsa-miR-20a-5p	UAAAGUGCUUAUAGUGCAGGUAG	23
hsa-miR-21-5p	UAGCUUAUCAGACUGAUGUUGA	22
hsa-miR-23b-5p	UGGGUUCCUGGCAUGCUGAUUU	22
hsa-miR-24-3p	UGGCUCAGUUCAGCAGGAACAG	22
hsa-miR-29a-5p	ACUGAUUUCUUUUGGUGUUCAG	22
hsa-miR-93-5p	CAAAGUGCUGUUCGUGCAGGUAG	23
hsa-miR-101-5p	CAGUUAUCACAGUGCUGAUGCU	22
hsa-miR-106a-5p	AAAAGUGCUUACAGUGCAGGUAG	23
hsa-miR-146a-5p	UGAGAACUGAAUUCCAUGGGUU	22
hsa-miR-150-5p	UCUCCCAACCCUUGUACCAGUG	22
hsa-miR-155-5p	UUAAUGCUAAUCGUGAUAGGGGU	23
hsa-miR-195-5p	UAGCAGCACAGAAAUAUUGGC	21
hsa-miR-221-5p	ACCUGGCAUACAAUGUAGAUUU	22
hsa-miR-221-3p	AGCUACAUUGUCUGCUGGGUUUC	23
hsa-miR-222-5p	CUCAGUAGCCAGUGUAGAUCCU	22
hsa-miR-483-5p	AAGACGGGAGGAAAGAAGGGAG	22
hsa-miR-486-5p	UCCUGUACUGAGCUGCCCCGAG	22