Circulating/Serum miR-22 a novel non-invasive predictor of poor clinical outcome in patients with diffuse large B-cell lymphoma: preliminary results of an on-going prospective study

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Background

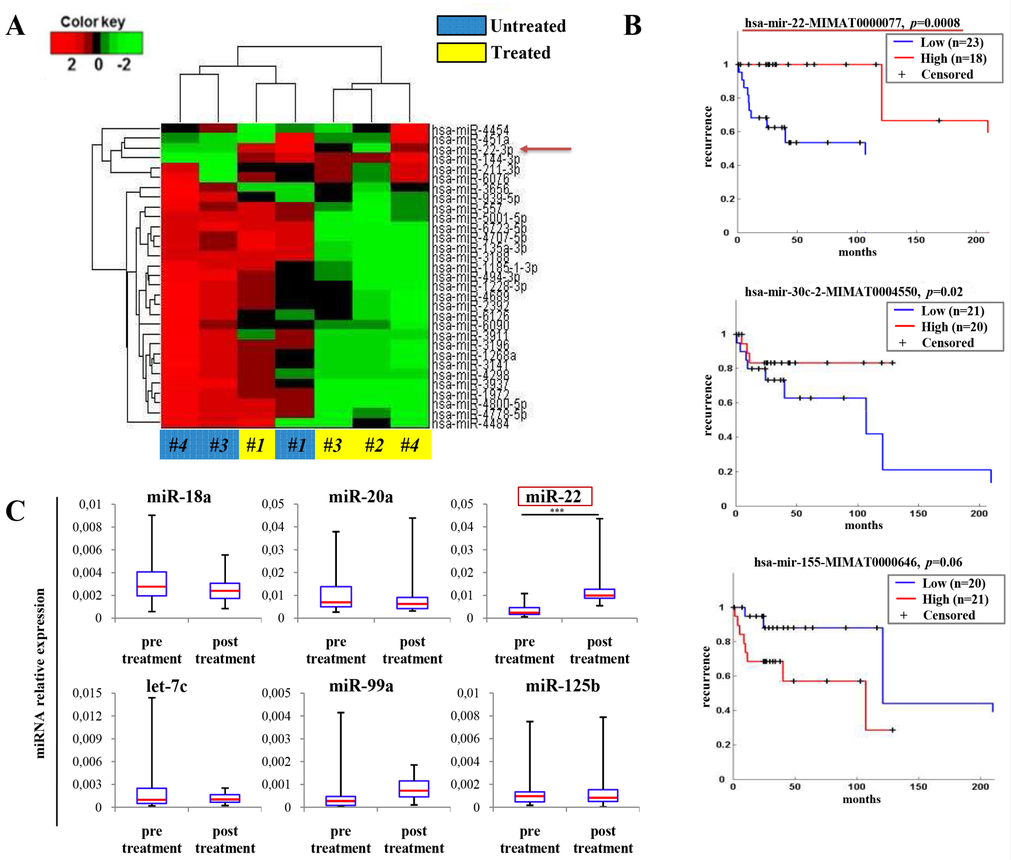
Diffuse large B-cell lymphoma (DLBCL), the most common high-grade non-Hodgkin lymphoma, is a heterogeneous group of tumors with aggressive clinical course. This heterogeneity makes prognostication and choice of treatment strategy difficult. Immunochemotherapy with rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone (R-CHOP) is the current first-line treatment. However, with this therapeutic approach up to 40% of patients still experience early treatment failure or relapse after initial response. Currently, only few biomarkers are able to predict resistance/response to therapy and are used in clinical practice.

MicroRNAs (miRNAs) are small non coding RNAs that can be released in body fluids in a very stable form, for this reason they are considered optimal candidates as non-invasive biomarkers for early diagnosis/follow-up of cancer patients. However, while tissue miRNAs in DLBCL patients have been extensively studied as novel diagnostic and prognostic biomarkers only few reports, to date, have evaluated the role of circulating miRNAs as potential prognostic factors. We have recently studied a small signature of circulating/serum miRNAs, including miR-22, previously shown to be associated with DLBCL. Our data suggest that serum miR-22 is interesting as potential prognostic biomarker, since its expression levels at diagnosis is correlated with progression free survival of DLBCL patients. Interestingly, through an integrative pathway analysis we shows that miR-22 target genes are involved in different important pathways such as p53 and PI3K/AKT signaling.

Aims

1) To validate the serum miR-22 as novel and reliable prognostic biomarker in DLBCL.
2) To dissect the role of miR-22 in the DLBCL pathogenesis, identifying the molecular pathways modulated by this microRNA.

1. Selection of miRNAs to evaluate as prognostic biomarkers for DLBCL patients

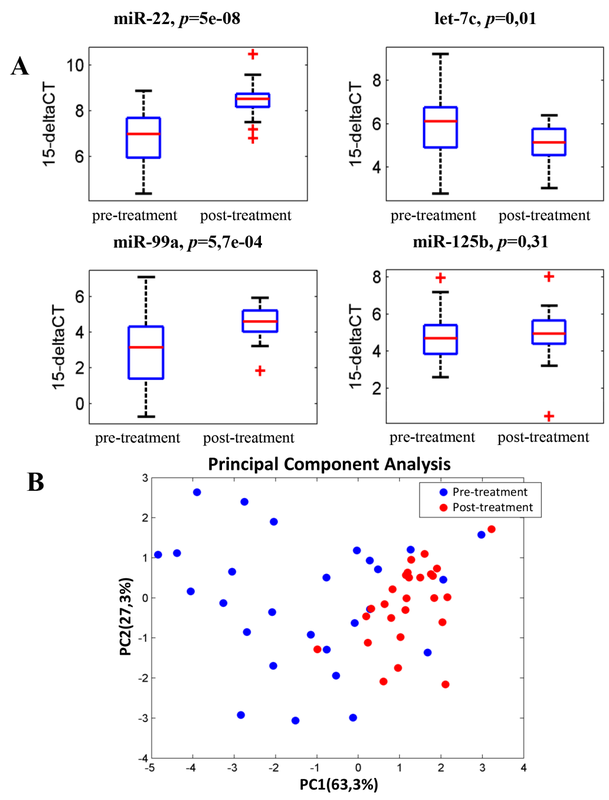


A Heat-map of hierarchical clustering of 31 selected miRNAs on serum of DLBCL patients at diagnosis and after R-CHOP treatment. Higher and lower values are represented by red and green points, respectively. **B** Kaplan-Meier PFS curves. Correlation between the indicated miRNAs and the rate of recurrence of DLBCL patients from TCGA data analysis. **C** Box-plot diagrams of relative miRNA expression levels, before/after treatment, in serum samples from a small cohort of DLBCL patients (n = 16), assessed by qRT–PCR. The expression levels of mature miRNAs were normalized to volume and UniSp2 spike-in RNA. Relative expression was calculated using the comparative ΔCt method. *** = p ≤ 0.001

Experimental Design

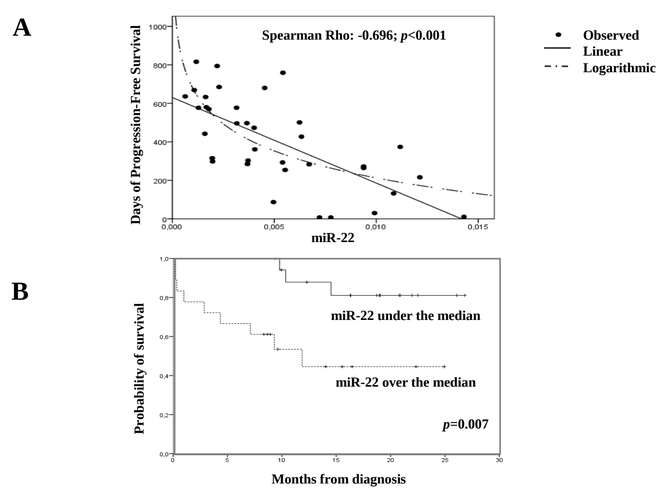
This is an on-going prospective study on newly diagnosed de novo DLBCL patients uniformly treated with 6 courses of R-CHOP. A small signature of miRNAs was selected using a triple selection criterion: (1) A microarray experiment using samples collected before and after treatment; (2) A bioinformatic analysis using data available in "The Cancer Genome Atlas" (TCGA) about miRNA levels in DLBCL tissue specimens with the aim of identifying miRNAs correlated with OS or PFS; from both these analyses miR-22 emerged as modulated after treatment and correlated with patients' survival. (3) Lastly, we evaluated the expression profiles of miR-22, identified by (1) and (2) approaches, and other miRNAs described to be involved in lymphoid malignancies. Again miR-22 expression levels are modulated before/after treatment. The Kaplan-Meier method was used in order to asses the correlation of miR-22 serum levels with the PFS of DLBCL patients. To investigate the molecular pathways modulated by miR-22 and potentially involved in the pathogenesis of DLBCL, we have performed an *in silico* target and pathway analysis on a DLBCL dataset available in TCGA.

2. Serum miR-22, let-7c and miR-99a are modulated after treatment in DLBCL patients



A Box-plot diagrams of relative miRNA expression levels in pre-treatment and post-treatment serum samples from DLBCL patients. **B** Principal Component Analysis plots for pre- and post-treatment samples considering the combination of miR-22, let7c and miR-99a serum levels.

3. Expression of serum miR-22 is predictive of PFS in DLBCL patients



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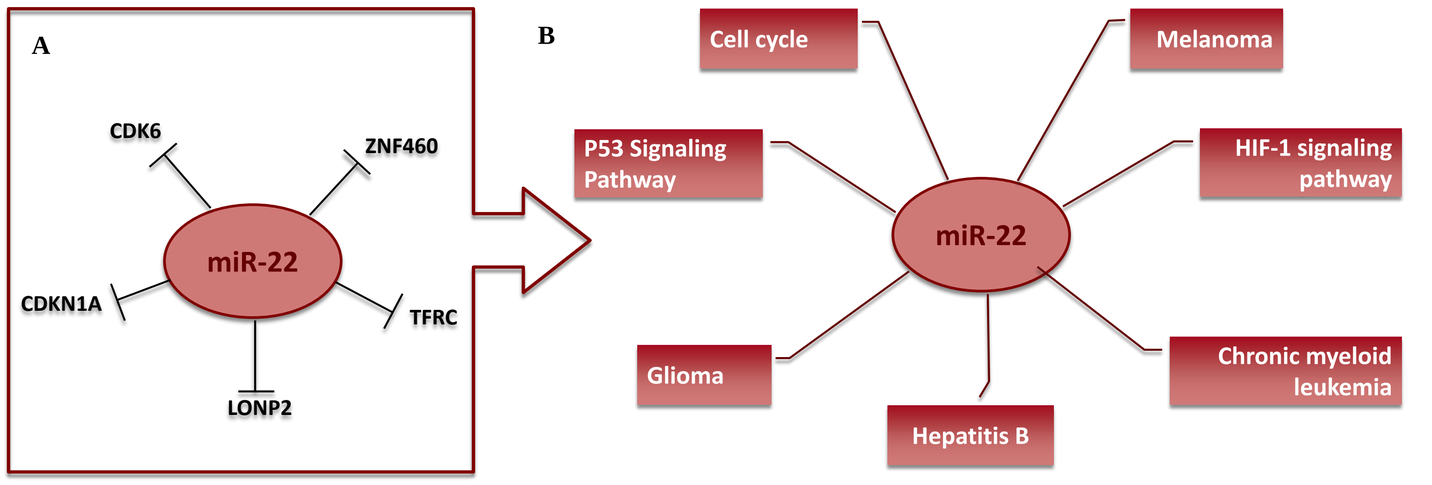
Factor	HR	95%CI	p-value
miRNA-22 (above vs. below median value)	5.19	1.38-19.56	0.015
IPI			
Intermediate vs. low	1.03	0.19-5.62	0.977
High vs. intermediate-low	2.07	0.67-6.48	0.209
Cell of origin (non-GC vs. GC)	0.42	0.13-1.38	0.153
Double hit (yes vs. no)	2.88	0.78-10.72	0.113

A Linear and logarithmic correlation between serum miR-22 values at diagnosis and days of PFS. **B** Kaplan–Meier curves for PFS in DLBCL patients with low and high expression of miR-22. **C** Progression Free Survival (PFS) analysis by Cox regression results. HR=hazard ratio; CI=confidence intervals; IPI=International Prognostic Index; GC= germinal-center; non-GC= non germinal center.

4. miR-22\mRNA: Integrative and Pathways Analysis in a TCGA/DLBCL dataset



A miR-22 validated target genes, which expression is inversely correlated to that of miR-22 in the TCGA dataset. **B** Pathways significantly enriched in miR-22 targets, identified trough the functional annotation clustering tool DAVID.

Conclusions

The identification of patient-specific miRNA expression profiles could be a useful tool to predict the response to standard chemo-immunotherapeutic treatment allowing a "personalized medicine" approach for these patients, with potential clinical advantages deriving from the BEST POSSIBLE THERAPY FOR THAT SPECIFIC BIOLOGICAL SUBSET OF PATIENTS. Our data suggest that expression level of serum miR-22 in DLBCL is associated with survival and is independent of the currently used clinical prognostic index IPI. In addition, the hierarchical clustering of targets and pathways, based on the levels of their interactions, can pave the way to the development of targeted therapy approaches for specific subgroups of DLBCL.