DOI: 10.1111/j.1600-0625.2012.01548.x
www.blackwellpublishing.com/EXD

Primary cutaneous anaplastic large cell lymphoma shows a distinct miRNA expression profile and reveals differences from tumor-stage mycosis fungoides

Marchina F. Benner[1], Erica Ballabio[2], Marloes S. van Kester[1], Nigel J. Saunders[3], Maarten H. Vermeer[1], Rein Willemze[1], Charles H. Lawrie[2,4,5,†] and Cornelis P. Tensen[1,†]

[1]Department of Dermatology, LUMC, Leiden, The Netherlands; [2]Lymphoid Malignancy Research Group, Nuffield Department of Clinical Laboratory Sciences, John Radcliffe Hospitals, University of Oxford, Oxford, UK; [3]Sir William Dunn School of Pathology, University of Oxford, Oxford, UK; [4]Biodonostia Research Institute, Paseo Doctor Begiristain s/n, San Sebastián, Spain; [5]IKERBASQUE, Basque Foundation for Science, Bilbao, Spain

Correspondence: Dr. C. P. Tensen, Department of Dermatology, T02-03, Leiden University Medical Center, Einthovenweg 20, 2333 ZC Leiden, The Netherlands, Tel.: +31 (0)715269369, Fax: +31 (0)715248106, e-mail: c.p.tensen@lumc.nl

[†]*These authors share senior authorship.*

Abstract: The miRNA expression profiles of skin biopsies from 14 primary cutaneous anaplastic large cell lymphoma (C-ALCL) patients were analysed with miRNA microarrays using the same control group of 12 benign inflammatory dermatoses (BID) as previously used to study the miRNA expression profile of tumor-stage mycosis fungoides (MF). We identified 13 differentially expressed miRNAs between C-ALCL and BID. The up-regulation of miR-155, miR-27b, miR-30c and miR-29b in C-ALCL was validated by miRNA-Q-PCR on independent study groups. Additionally, the miRNA expression profiles of C-ALCL were compared with those of tumor-stage MF. Although miRNA microarray analysis did not identify statistically significant differentially expressed miRNAs, miRNA-Q-PCR demonstrated statistically significantly differential expression of miR-155, miR-27b, miR-93, miR-29b and miR-92a between tumor-stage MF and C-ALCL. This study, the first describing the miRNA expression profile of C-ALCL, reveals differences with tumor-stage MF, suggesting a different contribution to the pathogenesis of these lymphomas.

Key words: miR-155 – miRNA microarray – mycosis fungoides – primary cutaneous anaplastic large cell lymphoma – Q-PCR

Accepted for publication 4 June 2012

Background

Primary cutaneous anaplastic large cell lymphoma (C-ALCL) is characterized by large cells with an anaplastic, pleomorphic or immunoblastic cytomorphology and by expression of the CD30 antigen by more than 75% of the tumor cells (1). Although its histology indicates an aggressive lymphoma, C-ALCL often shows an indolent clinical behaviour with a 10-year disease-specific survival (DSS) of approximately 90% (2–4). The molecular mechanisms involved in the development of this disorder are largely unknown (5).

MicroRNAs (miRNAs) are small non-coding RNA molecules that regulate gene expression posttranscriptionally. They may have oncogenic or tumor suppressing properties depending on their target genes (6,7). Several studies have demonstrated specific miRNA expression profiles in different types of CTCL suggesting a role in the pathogenesis of these disorders (8–12). For instance, recently, van Kester *et al.* (8) have elaborated on the miRNA expression profile of tumor-stage mycosis fungoides (MF), a CTCL with a 10-year DSS of 42%. So far, only one study focusing on the miRNA expression profiling of CTCL in general has included C-ALCL biopsies (10). Hence, the miRNA expression profile of C-ALCL is as yet unknown.

Questions addressed

The aim of this study was to determine the miRNA expression profile of C-ALCL compared with benign inflammatory dermatoses (BID) and with tumor-stage MF to identify miRNAs which may contribute to the pathogenesis of these malignancies.

Experimental design

For detailed material and methods, please see Supporting information (Data S1). Formalin fixed paraffin embedded (FFPE) skin biopsies containing more than 75% of CD30+ large cells were selected from 21 C-ALCL patients. In total, 14 biopsies were selected for array analysis, and seven additional biopsies were selected as a test group for validation by miRNA-Q-PCR. As a control group, FFPE biopsies of 12 benign inflammatory dermatoses (BID) containing T-cell-rich infiltrates were selected: five eczema and seven lichen planus cases for array analysis and five additional eczema and six lichen planus cases for miRNA-Q-PCR.

The complete miRNA profile of samples was elucidated using μRNA microarrays (8,14). The arrays were used within 3 months after printing. ANOVA analysis was performed to identify miRNAs differentially expressed between sample types, and multiple testing correction was done using the Benjamini–Hochberg method.

Results of miRNA microarray analysis of C-ALCL were compared with those recently published on 19 tumor stage MF (8), which were generated using the same platform (9).

MiRNA cDNA synthesis was performed as described before (8). MiRNA-Q-PCR was performed using Taqman miRNA assays and 2× Universal PCR mastermix (Applied Biosystems-Life Technologies, Carlsbad, CA, USA). All reactions were run on the LightCycler480 (Roche, Almere, the Netherlands), according to

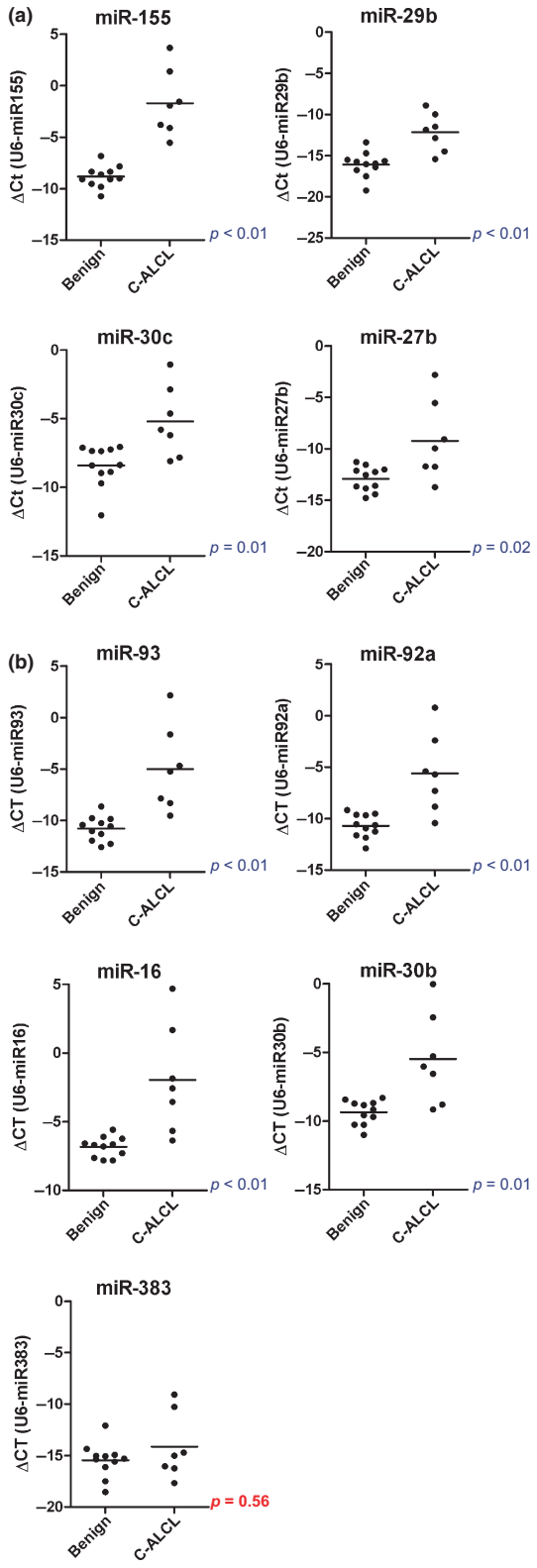

Figure 1. Expression levels of selected miRNAs as measured by miRNA-Q-PCR in an additional test group. Horizontal bars represent the mean. (a) Confirmation of four miRNAs selected for validation. (b) Four of five miRNAs additionally tested are significant differentially expressed between C-ALCL and benign controls (*P*-values measured by Mann–Whitney *U*-test).

manufacturer's protocol (Applied Biosystems). MiRNA expression levels were analysed using the ΔC_t method expressed relative to U6.

Results

MiRNA microarray analysis showed twelve miRNAs higher expressed and one miRNA lower expressed in C-ALCL compared with BID (adjusted $P < 0.05$; Table S1). To validate these results, miRNA-Q-PCR was performed for miR-155, miR-29b, miR-30c and miR-27b in an additional group of C-ALCL ($n = 7$) and BID (eczema $n = 5$ and lichen planus $n = 6$). All miRNA-Q-PCR results are consistent with the microarray data, showing significant differential expression between C-ALCL and BID ($P < 0.05$; Fig. 1a).

Comparison of miRNA microarray results from C-ALCL with those from tumor-stage MF showed differential expression of several miRNAs, but none remained statistically significant after

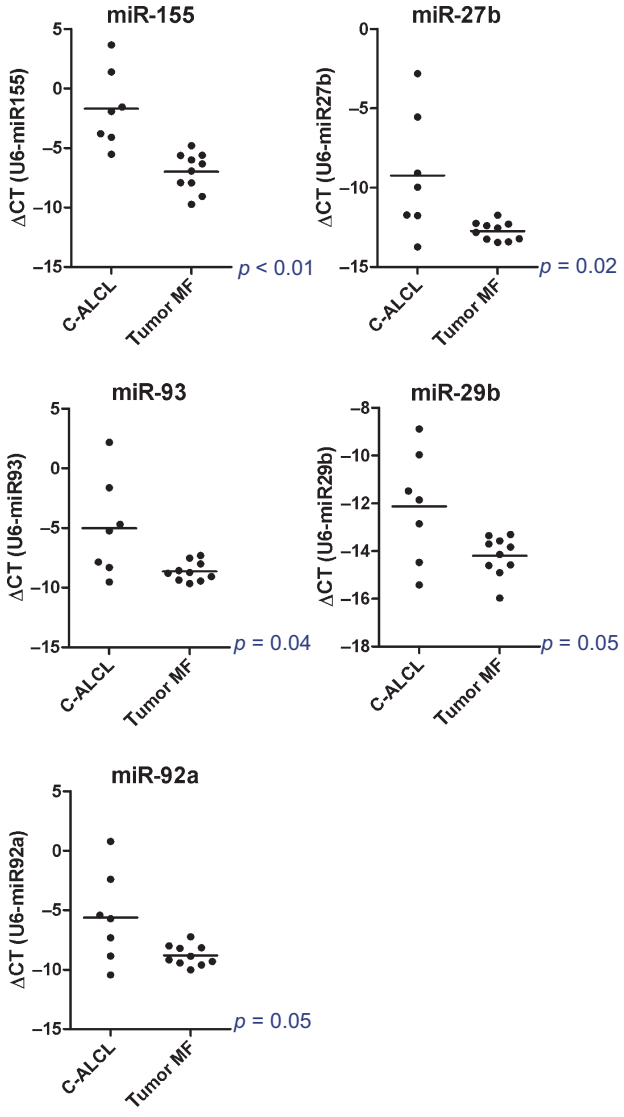

Figure 2. Expression levels of miR-155, miR-27b, miR-93, miR-29b and miR-92a as measured by miRNA-Q-PCR in C-ALCL and tumor-stage MF. Horizontal bars represent the mean (*P*-values measured by Mann–Whitney *U*-test).

multiple testing correction. However, we noticed that the set of miRNAs discriminating C-ALCL from benign controls (this study) is different from the set discerning tumor-stage MF from the same controls (8). We therefore extended the analysis of C-ALCL and used miRNA-Q-PCR to determine the expression of miR-93, miR-92a, miR-30b, miR-16 and miR-383, which were differentially expressed between tumor-stage MF and BID. Except for miR-383, all miRNAs showed significant up-regulation in C-ALCL vs. BID (Fig. 1b). In analogy, we also measured miR-29b and miR27b in tumor-stage MF, using the validation set as described by van Kester *et al.* (8), resulting in the up-regulation of miR-29b in tumor-stage MF vs. BID ($P < 0.01$), while miR-27b is not differentially expressed (data not shown). Finally, we determined whether any of the miRNAs assayed by miRNA-Q-PCR is differentially expressed between C-ALCL and MF. As depicted in Fig. 2, this analysis identified differential expression of miR-155, miR-27b, miR-93, miR-92a and miR-29b.

Conclusions

Using miRNA microarrays, we found thirteen miRNAs differentially expressed between C-ALCL and BID, twelve up-regulated and one down-regulated. We validated expression of miRNA-155, miR-27b, miR-30c and miR-29b with miRNA-Q-PCR in two independent groups of C-ALCL and BID. Because these array-based analyses of miRNA profiles of C-ALCL were performed using the same platform as the recently published experiments on miRNA profiling of tumor-stage MF, a proper comparison (including normalization and statistical analysis) was feasible (8,9). Under stringent statistical conditions, no differences between C-ALCL and MF were identified. However, the miRNA-Q-PCR-based analysis of an additional selection of individual miRNAs (chosen as being differentially expressed between tumor-stage MF or C-ALCL and BID) revealed not only further differences between the two disease entities (miR-155, miR-27b, miR-93, miR-92a and miR-29b) but also additional differences between C-ALCL and BID (miR-93, miR-92a, miR-27b, miR-30b and miR-16). Therefore, miRNA arrays are well suited for initial genome-wide screening, but apparently are less sensitive for detection of more subtle changes in miRNA expression. A similar observation was recently made by Ralfkiaer *et al.* (10) in the course of their miRNA profiling studies for diagnostic markers of CTCL who speculated that this might relate to cross-hybridization problems or a restricted dynamic range of miRNA microarrays. Moreover, miRNA microarrays are only capable of measuring the relative expression levels of known (annotated) miRNAs, and hitherto unknown miRNAs might be differentially expressed between the different disease entities as well. These restrictions might be overcome in the future using techniques like next generation deep sequencing, though the limited availability of suitable (frozen) material with a high tumor cell content will remain a challenge. The current miRNA expression profile of C-ALCL and the differences compared with tumor-stage MF provide a framework for further (functional) studies and may help to reveal the molecular pathogenesis of these lymphomas.

Acknowledgments

MvK, EB and MFB performed the research; MvK, EB, MFB, CPT and CHL analysed the data; MFB, CPT, CHL, MHV and RW wrote the paper. NJS contributed essential reagents and tools; MFB, MHV and RW collected patient samples; CHL and CPT designed the research study. The authors would like to thank WH Zoutman, JJ Out-Luiting (Department of Dermatology, LUMC), PA van der Velden (Department of Ophthalmology, LUMC) and RHAM Vossen (Center of Human and Clinical Genetics, LUMC) for their excellent assistance. This work was funded by grants from Netherlands Organization for Scientific Research (NWO) (MHV) and the Fondation René Touraine (MvK), and grants from the Leukaemia and Lymphoma Research (EB) and the Julian Starmer-Smith Memorial Fund (CHL).

Conflict of interests

The authors have declared no conflicting interests.

References

1 Willemze R, Jaffe E S, Burg G *et al.* Blood 2005: **105**: 3768–3785.
2 Bekkenk M W, Geelen F A, van Voorst Vader P C *et al.* Blood 2000: **15**: 3653–3661.
3 Benner M F, Willemze R. Arch Dermatol 2009: **145**: 1399–1404.
4 Liu H L, Hoppe R T, Kohler S *et al.* J Am Acad Dermatol 2003: **49**: 1049–1056.
5 Greisser J, Doebbeling U, Roos M *et al.* Exp Dermatol 2005: **14**: 380–385.
6 Lawrie C H. J Cell Moll Med 2008: **12**: 1432–1444.
7 Fabbri M, Croce C M. Curr Opin Haematol 2011: **18**: 266–272.
8 Van Kester M S, Ballabio E, Benner M F *et al.* Mol Oncol 2011: **5**: 273–280.
9 Ballabio E, Mitchell T, van Kester M S *et al.* Blood 2010: **16**: 1105–1113.
10 Ralfkiaer U, Hagedorn P H, Bangsgaard N *et al.* Blood 2011: **24**: 5891–5900.
11 Narducci M G, Arcelli D, Picchio M C *et al.* Cell Death and Disease 2011: **28**: 2.
12 Maj J, Jankowska-Konsur A, Sadakiewska-Chudy A *et al.* Br J Dermatol 2012: **166**: 331–336.
13 Van Doorn R, Van Haselen C W, van Voorst Vader P C *et al.* Arch Dermatol 2000: **136**: 504–510.
14 Lawrie C H, Saunders N J, Soneji S *et al.* Leukemia 2008: **22**: 1440–1446.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Differentially expressed miRNAs with chromosomal location.

Data S1. Material and methods.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.