Leukemia Research Reports 10 (2018) 52–54

Contents lists available at ScienceDirect

Leukemia Research Reports

journal homepage: www.elsevier.com/locate/lrr

Potentially important miRNAs in enteropathy-associated T-cell lymphoma pathogenesis: A pilot study

ARTICLE INFO

Keywords:
EATL
miRNA

Enteropathy associated T-cell lymphoma (EATL) is a rare form of Non Hodgkin Lymphoma occurring primarily in the intestinal tract and which arises from intra-epithelial T-lymphocytes (IELs) [1]. Previously known as Type 1 EATL, this lymphoma has a strong association with coeliac disease and occurs with a higher frequency in Northern Europe where coeliac disease is most prevalent. Morphologically the lymphoma cells primarily consist of medium-sized to large tumour cells with round or angulated vesicular nuclei, prominent nucleoli, and pale-staining cytoplasm and is often associated with a moderate to abundant reactive infiltrate of eosinophils, histiocytes, and small lymphocytes [2]. These lymphomas are characteristically CD56 negative but may express CD30. Prior genetic studies have shown that homozygosity for HLA-DQ2 (HLA-DB1*02) and allelic variants of the MYO9B gene region maybe associated with previously classified EATL and indeed, data from comparative genomic hybridization studies on tumour DNA suggest that chromosomal gains of 1q and 5q and segmental amplification of 9q or deletion in 16q are important in EATL pathogenesis [2]. Following the new WHO classification of lymphoid neoplasms Type 2 EATL has been reclassified as monomorphic epitheliotropic intestinal T-cell lymphoma (MEITL) [1]. MEITL is less commonly associated with coeliac disease and is characterized by a monomorphic infiltrate of small- to medium-sized lymphoma cells. CD30 is often negative in MEITL, and CD56 positivity suggests that a different mechanism underlies the lymphomagenic process of this tumour in contrast to EATL [2].

In EATL cases, there is often a variable time lapse between the initial diagnosis of coeliac disease and the onset of lymphoma and EATL is a significant cause of mortality in adult onset coeliac disease patients. Standard chemotherapeutic regimens with CHOP are associated with a cure rate of about 20% in EATL, though intensified therapy with autologous stem cell transplant have been associated with 5-year overall survival rates of 60% [3]. miRNAs are a class of small noncoding RNAs, approximately 22 nucleotides long that have been found to negatively regulate gene expression. Over 4500 miRNAs have been identified in humans and nearly all human protein encoding genes are controlled by miRNAs. They have been found to have roles in cell growth, differentiation, apoptosis and tumourigenesis [4]. No study to date has characterised miRNA expression in EATL.

In this pilot study we characterised the miRNA profile of a cohort of EATL from formalin fixed paraffin embedded tissue using TaqMan low density arrays targeting specifically miRNAs associated with T-cell neoplasia and normal T-cell function. Ten formalin fixed paraffin embedded cases of EATL classified according to WHO 2016 criteria [2] were identified from Irish patients in the surgical pathology files of St. James's Hospital and The Adelaide and Meath National Children's Hospital Tallaght Dublin (Fig. 1 and Supplemental Figure 1). Control tissue comprised duodenal biopsies from patients with no history of enteropathy ($n = 5$) and those with a history of untreated coeliac disease ($n = 4$). The relative expression of 95 miRNAs was quantified from extracted tumour RNA (the list of miRNA genes and the qPCR-based methodology is provided in Supplemental Table 1 and Supplemental Methods respectively).

Unsupervised hierarchical clustering of miR expression of the entire cohort showed that EATL samples formed a distinct cluster relative to the control group (Supplemental Fig. 2). To identify an EATL miR signature we performed comparative marker selection (http://genepattern.broadinstitute.org) and identified 13 downregulated miRs which distinguished EATL from the control group (Fig. 2 and Supplemental Table 2). We then assessed what pathways are potentially targeted by the 13 miR gene signature of EATL using KEGG pathway analysis. There were 571 validated gene targets for the 13 miR gene signature and several major target pathways were over-represented including the JAK/STAT, MAPK and PI3K-AKT pathways (Supplemental Figs. 3 and 4).

In this pilot study we demonstrate for the first time a potentially distinct miR signature associated with EATL and have identified several miRs that may potentially have a role in EATL pathogenesis. Interestingly all of the 13 miRs present in the EATL signature were downregulated indicating their role as potential tumour suppressor genes. Not surprisingly, we identified several pathways potentially affected by miR dysregulation in EATL, including several genes independently enriched in the JAK/STAT signalling pathway, which is frequently mutated in intestinal T-cell lymphoma highlighting its important role in EATL pathogenesis [5]. Indeed, with the development of new therapies and ongoing clinical trials, targeting both the JAK/STAT and MAPK pathway maybe worth considering in patients with EATL. Importantly several of the miRs

https://doi.org/10.1016/j.lrr.2018.10.002
Received 9 July 2018; Received in revised form 16 September 2018; Accepted 7 October 2018
Available online 16 October 2018

Fig. 1. Enteropathy Associated T cell lymphoma Clinicopathological Data, Immunophenotype and T-cell receptor Gene Rearrangement Analysis.

identified in the EATL signature are located in regions of chromosomal loss or gain that have been implicated in EATL pathogenesis previously (Supplemental Table 3). None of the downregulated miRs identified in the 13-gene signature map to chromosome 16q12.1 an area of genomic deletion previously identified in EATL.

In this pilot study we limited the repertoire of miRNAs that were interrogated following an extensive literature search. Whilst this introduced selection bias into the study it allowed us to focus on miRNAs relevant to normal T-cell function and malignancy. Several of the signature miRs have been characterised in a number of T-cell Non Hodgkin lymphomas and leukaemias including nodal peripheral T-cell lymphoma (ALCL, ATLL, AITL, T-ALL/T-LBL), cutaneous T-cell lymphoma and extranodal NK/T-cell lymphoma [6]. Interestingly, some of the signature miRs have been predictive of patient outcome in other haematological malignancies with miR-145 associated with worse prognosis in adult T-cell leukaemia. Indeed miR dysregulation in EATL maybe a result of epigenetic inactivation such as occurs frequently with miR-203 in other haematological malignancies.

Whole normal and enteropathic duodenal mucosa were used as controls in this series and whilst these samples may not accurately reflect the pre-neoplastic intra-epithelial lymphocyte (IEL) population, similar control material has been utilised previously. Further study is warranted on a large cohort of EATL cases interrogating all potential miRNAs (utilizing an unbiased technique such as RNA-Seq) to validate our observations and to analyse progressive miRNA dysregulation in microdissected IELs from normal duodenal mucosa, coeliac enteropathy (including refractory cases) relative to microdissected EATL cases.

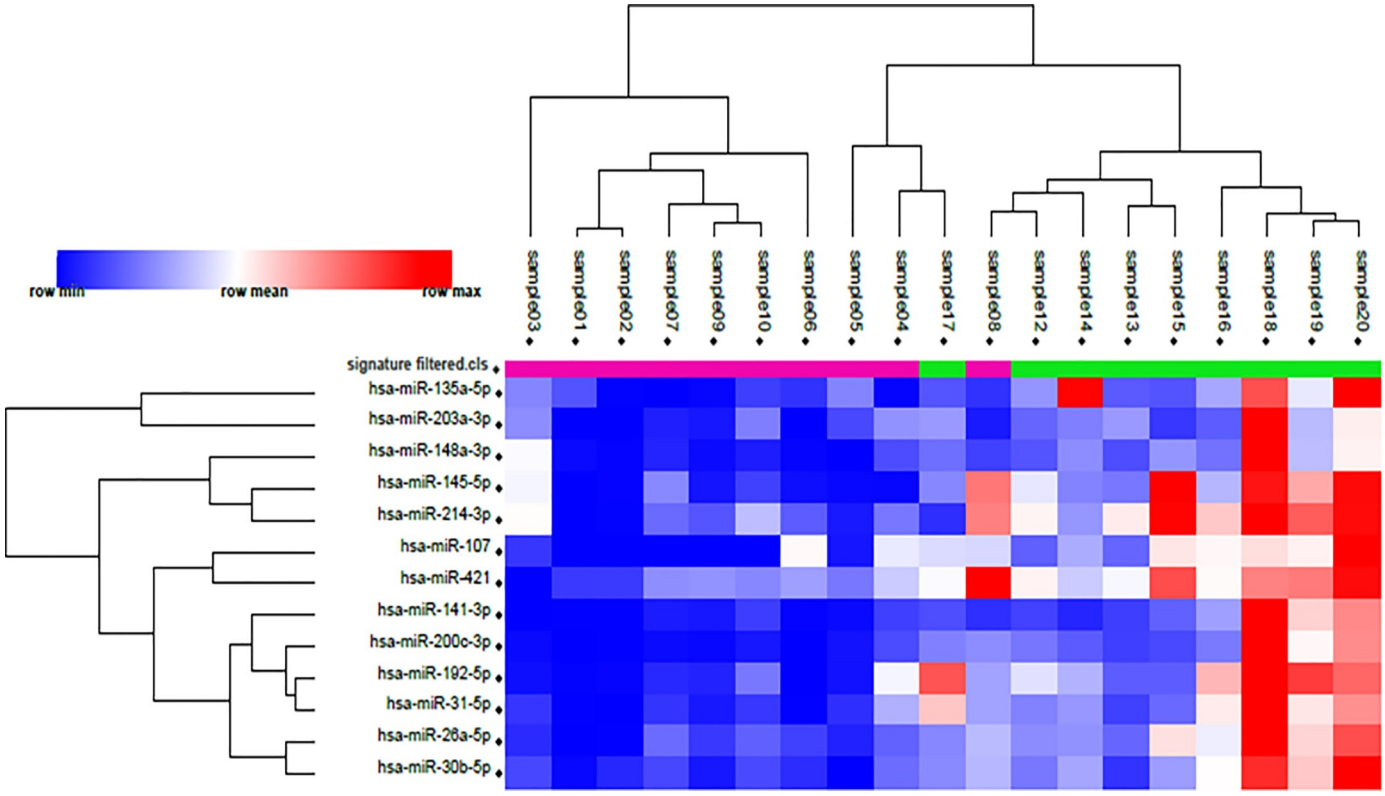

Fig. 2. Potential miR signature associated with EATL. A 13-miR classifier (all downregulated miRs) was identified by differential expression analysis. 9 EATL cases (magenta) form a distinct cluster with respect to both sets of duodenal controls (green = normal duodenum or coeliac disease) with 1 EATL case interspersed with the normal controls (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

Acknowledgments

We would like to thank Biobank Ireland Trust for their help with sample procurement.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.lrr.2018.10.002.

References

[1] PG Isaacson, A Chott, G Ott, et al., Enteropathy associated T-cell lymphoma, in: SH Swerdlow, E Campo, NL Harris, et al. (Eds.), WHO Classification of Tumors of Hematopoetic and Lymphoid Tissues, IARC, Ed. Lyon, 2017.
[2] A Di Sabatino, F Biagi, PG Gobbi, et al., How I treat enteropathy-associated T-cell lymphoma, Blood 119 (11) (2012) 2458–2468.
[3] M Sieniawski, N Angamuthu, K Boyd, et al., Evaluation of enteropathy-associated T-cell lymphoma comparing standard therapies with a novel regimen including autologous stem cell transplantation, Blood 115 (18) (2010) 3664–3670.
[4] A Lujambio, SW Lowe, The microcosmos of cancer, Nature 482 (7385) (2012) 347–355.
[5] A Nicolae, L Xi, TH Pham, et al., Mutations in the JAK/STAT and RAS signaling pathways are common in intestinal T-cell lymphomas, Leukemia 30 (11) (2016) 2245–2247.
[6] N Saki, S Abroun, M Soleimani, et al., Involvement of MicroRNA in T-Cell Differentiation and Malignancy, Int. J. Hemato.l Onco.l Stem. Cell Res. 9 (1) (2015) 33–49.

Lindsey Clarke[a,1], Raju SR Adduri[b,1], Paul Smyth[a,1], Fiona Quinn[c], Michael Jeffers[a], Barbara Dunne[a], John O'Leary[a], Susan McKiernan[d], Elisabeth Vandenberghe[c,e], Saumyadipta Pyne[f,g], Murali Dharan Bashyam[b], Orla Sheils[a], Richard Flavin[a,c,*]

[a] *Department of Histopathology, St. James's Hospital, Dublin and TTMI, Trinity College Dublin, Ireland*
[b] *Laboratory of Molecular Oncology, Centre for DNA Fingerprinting and Diagnostics, Nampally, Hyderabad, India*
[c] *Department of Cancer Molecular Diagnostics, St. James's Hospital, Dublin, Ireland*
[d] *Department of Gastroenterology, St. James's Hospital, Dublin, Ireland*
[e] *Department of Haematology, St. James's Hospital, Dublin, Ireland*
[f] *Public Health Dynamics Laboratory, and Department of Biostatistics, Graduate School of Public Health, University of Pittsburgh, PA, USA*
[g] *ICMR National Institute of Medical Statistics, New Delhi, India*
E-mail address: rflavin@stjames.ie (R. Flavin).

[*] Corresponding author at: Department of Histopathology, St. James's Hospital, Dublin and TTMI, Trinity College Dublin, Ireland
[1] Joint First Authors.