The microRNA genome in Diffuse Large B-cell Lymphoma

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Abstract

MicroRNAs (miRNAs) are small non-protein coding RNAs, that attenuate gene expression by pairing to the 3'UTR of target transcripts inducing RNA cleavage or translational inhibition. This novel class of genes is believed to regulate the expression of ~ 1/3 of the human genome. Expectedly therefore, a critical role for miRNAs in several physiologic and pathological processes, including cancer, has been recently uncovered. However, the role of miRNAs in the most common type of lymphoid malignancies in adults, Diffuse large B-cell lymphoma (DLBCL), has not been studied. To address this issue, we designed and implemented a tiling array CGH platform that defines the structural integrity of all human miRNAs in a single assay. Using this tool we analyzed a large series of 85 well characterized DLBCLs and found disruption of at least one miRNA locus in 95% of tumors. This data suggests that miRNAs may play a role in the pathogenesis of DLBCL.

Introduction

Diffuse large B-cell lymphoma (DLBCL) is an aggressive tumor that derives from mature B-lymphocytes located within the lymph nodes (1). DLBCL is a common and often fatal cancer. The cause of DLBCL is unknown, but many genetic abnormalities have been associated with its development and progression. Indeed, a large fraction of DLBCLs exhibit recurring chromosomal translocations and DNA imbalances leading to aberrant gene expression.

MicroRNAs (miRNAs) are small non-protein coding RNAs, that attenuate gene expression by pairing to the 3'UTR of target transcripts inducing RNA cleavage or translational inhibition (2). Recently, a link between miRNA disruption and cancer was suggested. However, a complete map of the structural integrity of the miRNA genome in DLBCL is not available. To address this issue in a comprehensive and high-throughput manner we designed a unique oligo-based tilling array platform that covered at high resolution all human miRNA loci.

Herein, we describe the implementation of this platform to successfully create a comprehensive atlas of the miRNA genome in a large series of well-characterized DLBCLs. The data will start to delineate the contribution of miRNAs to DLBCL's pathogenesis.

Materials and Methods

Array design:

An Oligonucleotide Array-based CGH (oaCGH) platform was created to analyze the structural integrity of all miRNA genes across the human genome. The miRNA data used in the analysis was retrieved from miRBase Registry (version 9.1, February 2007) (http://microrna.sanger.ac.uk/sequences/index.shtml). Each array (printed with Agilent

technology) contained 44.000 probes covering at high density (~500bp interval) the 20 kb situated immediately upstream or downstream from the mature miRNA sequence for each one of the 471 miRNA loci in the human genome. In addition, approximately 1200 “backbone” probes were randomly distributed throughout the genome.

Sample Preparation

A total of 89 samples were used. Four of the samples were controls (pooled DNA derived from peripheral blood cells from normal individuals), 60 were primary tumor samples and 25 were derived DLCBL cell line samples. The samples were prepared following the Agilent (array manufacturer) protocols. In brief, high molecular weight DNA was isolated using the Maxwell 16 DNA purification kits (Promega), digested with restriction enzymes, fluorescently labeled (Agilent Genomic DNA Labeling Kit PLUS) and hybridized to the arrays. After overnight hybridization, the arrays were washed and scanned using the Agilent DNA microarray scanner. Subsequently, the scanned images (fluorescence intensity) were converted to numerical values using Agilent’s Feature Extraction Software.

Data analyses:

The array design file and the extracted data were uploaded in the CGH Analytics 3.4.40 software (Agilent) centralized (to ensure that the zero-point reflects the most-common-ploidy state), normalized (where the expected average is subtracted from all log ratios, and these modified log ratios are divided by the estimated variance). The quality of the experiment was defined by a series of control metrics, including, derivative log ratio (DLR) spread, signal intensity, signal to noise ratio, background noise and spike-in reference. In this study, tumor/reference DNA fluorescent intensity ratios <0.5 or >1.5 were considered as alteration (Figures 1 and 2) and copy number estimates were made with the aberration detection algorithm, ADM-2. ADM-2 identifies all aberrant intervals in a given sample defined by consistently high or low log ratios of sample/reference signals based on the statistical score of the interval. The statistical score is computed as the deviation of the absolute average of the normalized log ratios of probes within a certain interval (which can be manually defined) from its expected value of zero. Furthermore, quality information about each probe measurement (using log-ratio error information) is included. Thus the final score takes into account both absolute average log ratio and the number and quality of the probes in the preset analysis interval.

CGH Analytics also provides a visual representation of the miRNAs across the entire human genome. This feature allowed for an extensive manually curated examination of all 471 loci in the 85 tumor samples including in this project (see results).

Results and Discussion

Disruption of miRNA genes is widespread in DLBCLs. Of the 60 primary tumors studied 57 showed alterations targeting at least one miRNA locus; all 25 cell lines studied had aberration targeting the miRNA genes. In addition, multiple miRNA genes are targeted for gain or loss in a single tumor (an average of 61 per tumor), a phenomenon reminiscent of the multiple and cooperative disruption of classical mRNA genes and tumor development. Globally, there was no clear bias towards the type of aberration detected: of the 471 miRNA analyzed 435 were deleted and 426 amplified in at least one case. However, for those miRNA found to be frequently targeted (>20% of the cases studied) –amplification appears to be a more consistent feature. Forty eight miRNA genes were found to be amplified in more than 20% of cases whereas only seven miRNAs (including the B-cell relevant miR-16) were deleted in similar percentage of cases. There was significant overlap between the aberrations found in primary tumors and cell lines – especially concerning the miRNA genes targeted for deletion – suggesting that these lines can be used as models to understand the functional contribution of these miRNA towards tumor development.

These results suggest that miRNAs may contribute to DLBCL pathogenesis. The data obtained with this investigation will guide further studies aimed at defining the relationship between the structural abnormalities and differential miRNA expression in DLBCL.

Tables and Figures

CGH Analytics Gene View

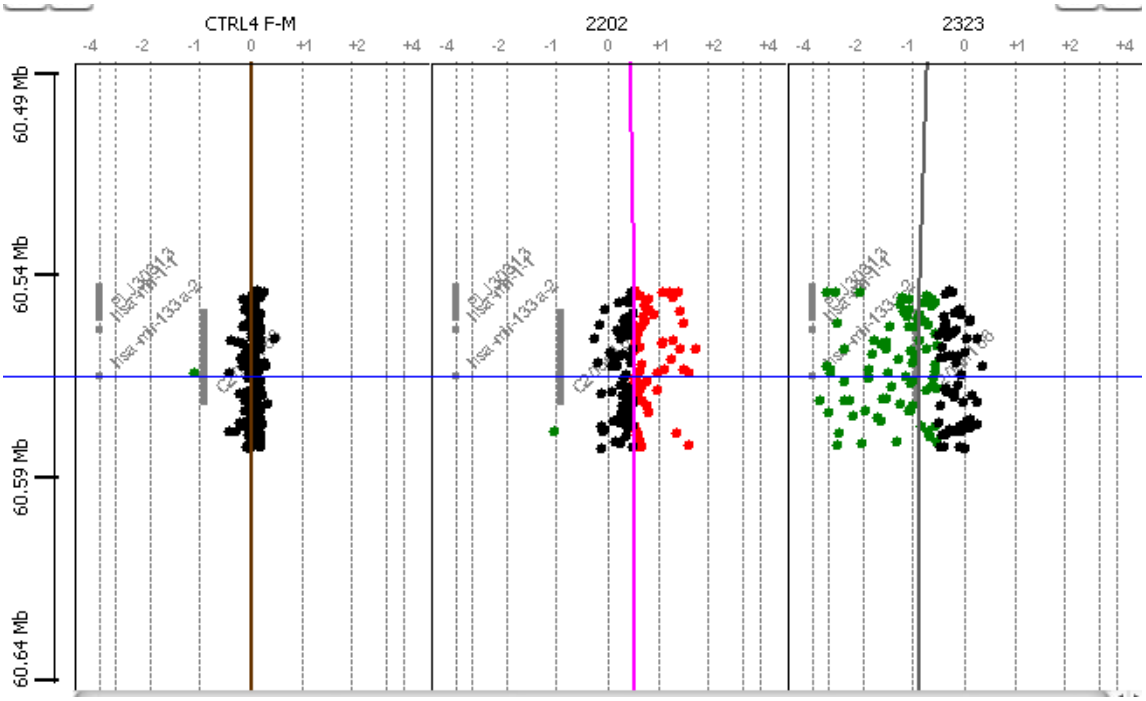

Figure 1. View of miR-133a-2 for 2 tumor samples: 2202 and 2323, and a control on CGH analytics. The control has no gain or deletion on miR-133a-2 which is represented by the array of only black probes. 2202 shows a gain on miR-133a-2 using a .5 cutoff which is represented by the red probes.2323 shows a deletion on miR-133a-2 using a .5 cutoff which is represented by the green probes.

CGH Analytics Chromosome View

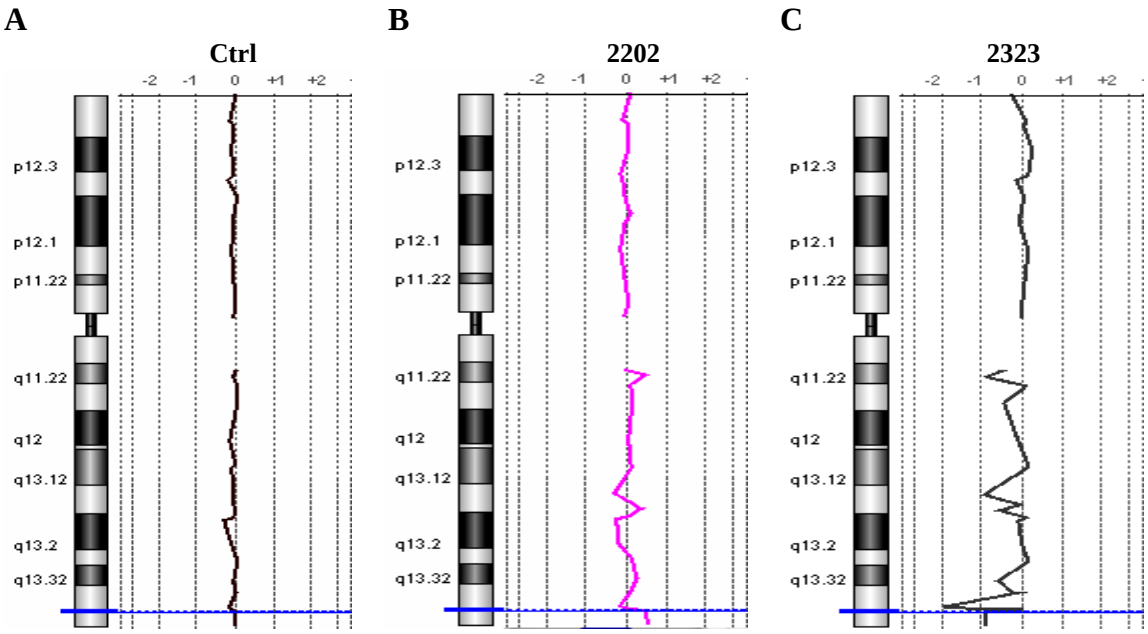

Figure 2. Chromosome view of Control (A), 2202 (B) and 2323 (C) on Chromosome 20. The Blue line represents miR-133a-2. The spikes in the colored lines going down the chromosome indicate gains or deletions in that area of the chromosome. Spikes in a positive direction at .5 or beyond are considered gains and spikes in a negative direction at .5 or beyond is considered a deletion. (Notice the control is fairly steady and there are many spikes in the tumor samples)

A

miRNAs that have Alterations in at least 20% of Samples

ID	Chromosome	% Alterations	ID	Chromosome	% Alterations	ID	Chromosome	% Alterations
hsa-mir-200b	1	34.12	hsa-mir-216	2	25.88	hsa-mir-574	4	22.35
hsa-mir-200a	1	34.12	hsa-mir-560	2	25.88	hsa-mir-575	4	22.35
hsa-mir-429	1	34.12	hsa-mir-128a	2	25.88	hsa-mir-576	4	22.35
hsa-mir-551a	1	34.12	hsa-mir-10b	2	25.88	hsa-mir-367	4	21.18
hsa-mir-34a	1	31.76	hsa-mir-561	2	24.71	hsa-mir-302d	4	21.18
hsa-mir-801	1	31.76	hsa-mir-26b	2	24.71	hsa-mir-302a	4	21.18
hsa-mir-552	1	30.59	hsa-mir-375	2	24.71	hsa-mir-302c	4	21.18
hsa-mir-30e	1	30.59	hsa-mir-153-1	2	24.71	hsa-mir-302b	4	21.18
hsa-mir-30c-1	1	29.41	hsa-mir-562	2	24.71	hsa-mir-577	4	21.18
hsa-mir-101-1	1	29.41	hsa-mir-149	2	24.71	hsa-mir-578	4	21.18
hsa-mir-186	1	29.41	hsa-mir-563	3	24.71	hsa-mir-579	5	21.18
hsa-mir-137	1	28.24	hsa-mir-128b	3	24.71	hsa-mir-580	5	21.18
hsa-mir-553	1	28.24	hsa-mir-26a-1	3	24.71	hsa-mir-581	5	21.18
hsa-mir-197	1	27.06	hsa-mir-138-1	3	24.71	hsa-mir-449	5	21.18
hsa-mir-554	1	27.06	hsa-mir-564	3	24.71	hsa-mir-449b	5	21.18
hsa-mir-92b	1	27.06	hsa-mir-565	3	24.71	hsa-mir-582	5	21.18
hsa-mir-555	1	27.06	hsa-mir-425	3	24.71	hsa-mir-9-2	5	21.18
hsa-mir-9-1	1	27.06	hsa-mir-191	3	24.71	hsa-mir-583	5	21.18
hsa-mir-765	1	27.06	hsa-mir-566	3	24.71	hsa-mir-584	5	20.00
hsa-mir-556	1	27.06	hsa-let-7g	3	24.71	hsa-mir-143	5	20.00
hsa-mir-557	1	27.06	hsa-mir-135a-1	3	24.71	hsa-mir-145	5	20.00
hsa-mir-214	1	27.06	hsa-mir-567	3	24.71	hsa-mir-378	5	20.00
hsa-mir-199a-2	1	27.06	hsa-mir-568	3	24.71	hsa-mir-146a	5	20.00
hsa-mir-488	1	27.06	hsa-mir-198	3	24.71	hsa-mir-103-1	5	20.00
hsa-mir-181b-1	1	27.06	hsa-mir-15b	3	23.53	hsa-mir-218-2	5	20.00
hsa-mir-181a-1	1	27.06	hsa-mir-16-2	3	23.53	hsa-mir-585	5	20.00
hsa-mir-135b	1	25.88	hsa-mir-551b	3	23.53	hsa-mir-340	5	20.00
hsa-mir-29c	1	25.88	hsa-mir-569	3	23.53	hsa-mir-548a-1	6	20.00
hsa-mir-29b-2	1	25.88	hsa-mir-28	3	22.35	hsa-mir-219-1	6	20.00
hsa-mir-205	1	25.88	hsa-mir-570	3	22.35	hsa-mir-586	6	20.00
hsa-mir-215	1	25.88	hsa-mir-571	4	22.35	hsa-mir-206	6	20.00
hsa-mir-194-1	1	25.88	hsa-mir-95	4	22.35	hsa-mir-133b	6	20.00
hsa-mir-558	2	25.88	hsa-mir-572	4	22.35	hsa-mir-30c-2	6	20.00
hsa-mir-559	2	25.88	hsa-mir-218-1	4	22.35	hsa-mir-30a	6	20.00
hsa-mir-217	2	25.88	hsa-mir-573	4	22.35	hsa-mir-587	6	20.00

B

miRNAs that have Gains in at least 20% of Samples

ID	Chromosome	% Gain	ID	Chromosome	% Gain	ID	Chromosome	% Gain
hsa-mir-200b	1	31.76	hsa-mir-553	1	22.35	hsa-mir-181b-1	1	20.00
hsa-mir-200a	1	31.76	hsa-mir-197	1	22.35	hsa-mir-181a-1	1	20.00

C

miRNAs that have Gains in at least 20% of Samples

ID	Chromosome	% Deletions	ID	Chromosome	% Deletions	ID	Chromosome	% Deletions
hsa-mir-588	6	29.41	hsa-mir-548a-2	6	21.18	hsa-mir-573	4	20.00
hsa-mir-650	22	29.41	hsa-mir-16-1	13	21.18	hsa-mir-597	8	20.00
hsa-mir-31	9	23.53	hsa-mir-15a	13	21.18			
hsa-mir-383	8	22.35	hsa-mir-218-1	4	20.00			

Figure 4.

Acknowledgements

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References

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2. Bartel, David P. 2004. MicroRNAs: Genomics, Biogenesis, Mechanism, and Function. Cell. **116**: 281-297.2.