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MicroRNA Expression in Follicular Lymphoma

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1. Introduction

Lymphoma is the fifth most common cancer type in the Western world, accounting for approximately 12,000 cases per annum in the UK alone. Moreover the occurrence of this type of cancer has been increasing. The age-adjusted incidence of non-Hodgkin's lymphoma (NHL) in the US for example has increased 74% between 1976 and 2001 (SEER 2005). Follicular lymphoma (FL) is the most common form of low grade B-cell lymphoma (75-80% of all cases) representing about a third of all NHL cases in the US, and a quarter of all cases in Europe (Anderson et al. 1998). FL is characterised by the presence of the t(14;18) translocation in 90% of patients associated with up-regulation of the anti-apoptotic protein BCL2. Whilst FL tumours are chemo-sensitive, the disease is essentially incurable, with patients following a relapsing-remitting clinical course, typically experiencing several episodes of disease before eventually becoming refractory to treatment.

Although indolent, with a median overall survival (OS) of ~10 years, about 30% of FL patients undergo high-grade transformation to an aggressive lymphoma that is histologically indistinguishable from diffuse large B-cell lympohoma (DLBCL). Transformed FL (tFL) patients have a particularly poor outcome with a median survival of <14 months (Wrench et al. 2010). The molecular basis of FL transformation is only poorly understood and importantly to date there are no reliable biomarkers that can identify FL patients at risk of transformation. In this chapter we will review the experimental evidence for the involvement of microRNAs in the pathology of FL with particular focus on the transformation process.

2. Follicular lymphoma

FL is a neoplasm of follicle center B cells (centrocytes) characterized by a (partially) follicular growth pattern. FL tumor cells are believed to arise from normal germinal centre–associated (GC) B cells as they express the same antigen profile as GC B cells (i.e. CD19, CD20, CD10, BCL6, and membrane-bound IgM or IgG), share many morphological features of normal GC cells, and are found within a follicular architecture embedded in a network of T cells and follicular dendritic cells. In contrast to normal GC cells however, FL tumour cells are characterised by the presence of t(14:18) translocation resulting in expression of the anti-

apoptotic molecule BCL2. This translocation is detectable in approximately 75% of cases by traditional karyotyping techniques but in over 90% of cases measured by polymerase chain reaction (PCR) (Tsujimoto et al. 1985). Although considered to be an essential feature of FL pathology, this genetic insult appears not to be in itself sufficient to cause FL as *BCL2* transgenic mice do not readily develop lymphoma (McDonnell & Korsmeyer 1991; Strasser et al. 1993). Furthermore, the t(14;18) translocation is not an uncommon finding in normal B cells, being detectable in over 50% of healthy individuals (Roulland et al. 2006). Therefore, whilst the presence of t(14:18) is highly suggestive of FL, it is by no means diagnostic, and indeed may be found in other apparently unrelated cases of NHL including 15-30% of DLBCL cases (Iqbal et al. 2004). Furthermore about 5% of FL cases lack the t(14:18) translocation instead being characterised by a BCL6 translocation t(3;14) and displaying an almost exclusive centroblastic morphology (Jaffe et al. 2001).

FL predominantly affects adults with a median age of 59 years and a male:female ratio of 1:1.7 (Anderson et al. 1998). Most FL patients already have widespread disease at time of presentation, predominantly in the lymph nodes, but FL may also involve the spleen, bone marrow, and peripheral blood and occasionally extra-nodal sites such as the gastro-intestinal tract or skin. Patients may be asymptomatic with slowly progressive lymphadenopathy or present with symptomatic complications of advancing tumour growth that require treatment. In nearly all survival studies, despite initial responsiveness to treatment, most patients relapse, and will eventually die of their disease.

2.1 Histological transformation of FL tumors

A percentage of FL patients (10-60% depending on the study) will eventually undergo high grade transformation from indolent FL to a much more aggressive tumor that is histologically indistinguishable from DLBCL, and is associated with a much poorer prognostic outcome. A recent study of 325 patients (median follow-up 15 years) found the risk of transformation to be 28% (Montoto et al. 2007). Despite the use of high dose therapy for transformed FL (tFL) cases, response rates are still lower than histologically equivalent *de novo* cases of DLBCL with a median survival of just 1.2 years. The molecular mechanisms behind this phenomenon, however, are very poorly understood and consequently the identification of at-risk patients, who might benefit from up-front high dose treatment modalities, remains one of the greatest challenges facing onco-hematologists today.

Lossos *et al* identified 671 genes that were aberrantly expressed in at least three of twelve paired biopsy samples which fell into two distinct groups; those that had *c-myc* and its target genes up-regulated and those where these genes were down-regulated (Lossos et al. 2002). Another study of five paired samples identified 36 up-regulated and 66 down-regulated genes, seven of which were common with the study of Lossos *et al* (de Vos et al. 2003). Sixty-seven and 46 genes were found to be up-regulated and down-regulated respectively in a series of eleven paired samples analyzed by Elenitoba-Johnson *et al* (Elenitoba-Johnson et al. 2003). Up-regulation of p38BMAPK was confirmed immunohistochemically as it was detected in DLBCL cases but not FL or normal GC cells. Davies *et al* examined the gene expression profile of twenty paired lymphoma samples taken pre- and post-transformation (Davies et al. 2007). They found that transformation proceeded by at least two molecular pathways; one characterized by a cell proliferation signature that was associated with recurrent oncogenic abnormalities and a decrease in T cell and follicular dendritic cell genes, while the other group showed no increase in

proliferation genes and followed an as yet undetermined route. In contrast a gene expression study of non-paired patients (24 FL patients who underwent transformation, 22 FL patients without transformation (after 7 years) and 24 DLBCL patients who had previously transformed from FL) found that gene expression was too heterogeneous to reliably predict transformation (Glas et al. 2007). They did however report a correlation by immunohistochemistry with the spatial distribution to neoplastic follicles and the activation of $CD4^+$ T cells and specifically T-helper 1 cells ($P>0.05$). They did not find any correlation with other infiltrating cell populations including $CD68^+$ macrophages or regulatory T cells.

Additionally, genomic alterations have been demonstrated to be associated with transformation of FL. The acquisition of novel mutations in PIM-1, PAX-5, RhoH/TTF and c-MYC genes, due to aberrant somatic hypermutation, was found in 5/9 cases that had undergone transformation (Rossi et al. 2006). Genomic aberrations were found to be more common in transformed cases of DLBCL than non-transformed FL and the alterations -6q16-21 and +7pter-q22 were only found in transformed DLBCL but not in follicular lymphoma whereas -4q13-21 was more common in transformed than *de novo* DLBCL (Berglund et al. 2007).

However, despite intensive research the molecular basis for transformation in FL patients remains largely unknown. Recently, ourselves and others, have raised the possibility that microRNAs may be important factors in both FL transformation and antecedent FL lymphogenesis (Roehle et al. 2008; Lawrie et al. 2009).

3. MicroRNAs

MicroRNAs are a recently discovered class of naturally occurring short non-coding RNA molecules that regulate eukaryotic gene expression post-transcriptionally. There are now more than 900 human microRNAs that have been identified through cloning and/or sequence analysis (miRBase- (Griffiths-Jones et al. 2006)), and it is believed some 60% of all human genes are a target for microRNA regulation (Friedman et al. 2009). MicroRNAs have been shown to play key regulatory roles in virtually every aspect of biology including developmental timing, cell differentiation, apoptosis, cell proliferation, metabolism organ development, and hematopoiesis (Kim 2005). The potential importance of microRNAs in cancer is implied by the finding that the majority of human microRNAs are located at cancer-associated genomic regions (Calin et al. 2004), and there is now overwhelming evidence that dysfunctional expression of microRNAs is a common, if not ubiquitous, feature of cancer in general and lymphoid malignancy in particular (Lawrie 2008; Iorio & Croce 2009).

Despite the fundamental role that microRNAs appear to play in biology, these molecules were unknown to the scientific world until 1993 when *lin-4*, a *C. elegans* developmental regulator was identified (Lee et al. 1993; Wightman et al. 1993). The significance of this finding was not however realised until seven years later when another worm microRNA, *let-7* was discovered (Reinhart et al. 2000). Unlike *lin-4*, the sequence of *let-7* was found to be highly conserved in almost all organisms (Pasquinelli et al. 2000). It was soon realised that similar sequences were scattered throughout eukaryotic genomes that were first called microRNAs in 2001 (Lee & Ambros 2001).

3.1 MicroRNA biosynthesis and function

The majority of human microRNAs are encoded within introns of coding or non-coding mRNAs whilst others are located exgenically, within the exons of non-coding mRNAs or

within the 3'UTR sequence of mRNA (Rodriguez et al. 2004). MicroRNAs are transcribed as 5'-capped large polyadenylated transcripts (pri-microRNA) primarily in a Pol II-dependent manner (Figure 1), although the involvement of Pol-III transcription has also been postulated for microRNAs encoded within Alu repeat sequences (Borchert et al. 2006). Approximately 40% of human microRNAs are co-transcribed as clusters encoding up to eight distinct microRNA sequences in a single pri-microRNA transcript (Altuvia et al. 2005; Hertel et al. 2006). Pri-microRNAs are cleaved within the nucleus by Drosha, an RNaseIII-type nuclease, to form 60-70 nucleotide hairpin structures (pre-microRNA). Drosha by itself possesses little enzymatic activity and requires the cofactor DiGeorge syndrome critical region 8 gene (DGCR8) in humans (Pasha in *Drosophila*) to form the so-called microprocessor complex (Yeom et al. 2006). Once produced, the pre-microRNAs are

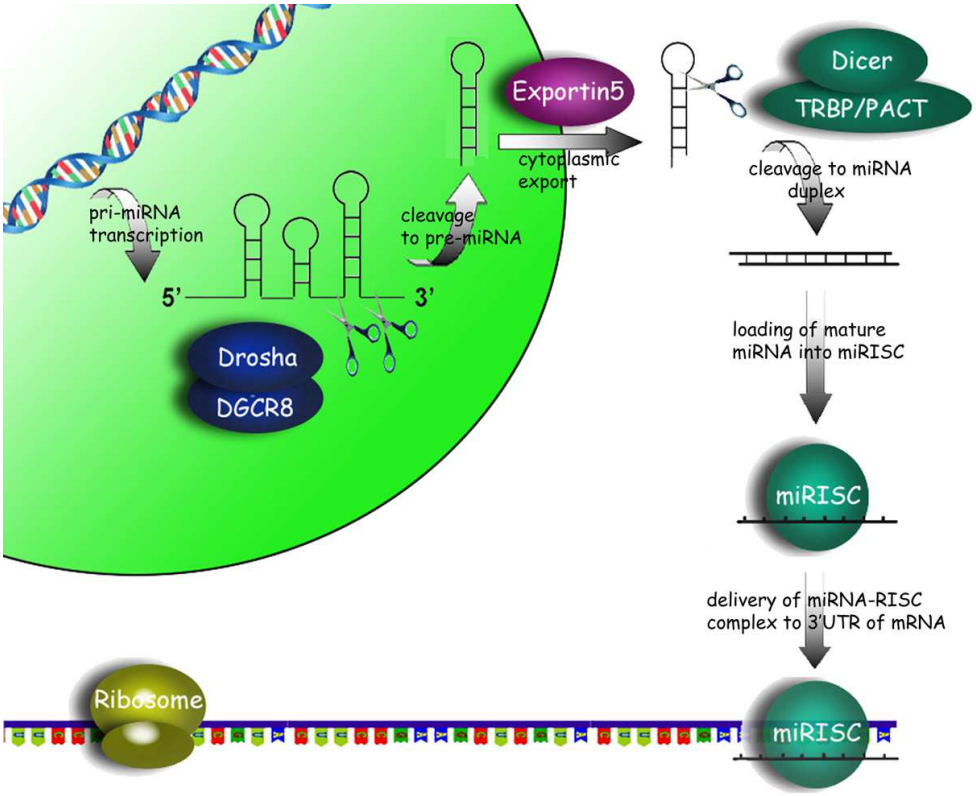

Fig. 1. **Schematic diagram of microRNA biosynthesis and function in animal cells.** Pri-microRNA precursor is transcribed in Pol-II dependent manner and then cleaved by microprocessor complex (Drosha/DGCR8) to form hairpin-structure pre-microRNA. Pre-microRNAs are exported from the nucleus by exportin-5 in a RAN-GTP dependent manner where they are cleaved into an asymmetric duplex by action of Dicer and accessory proteins. The mature microRNA is loaded into the miRISC complex which binds to cognate 3'UTR sequence of target mRNA resulting in either degradation of mRNA, or to blockage of translation without mRNA degradation.

exported from the nucleus to the cytoplasm by Exportin5 in a Ran-GTP dependent manner (Zeng 2006). The cytoplasmic pre-microRNA is further cleaved to form an asymmetric duplex intermediate (microRNA: microRNA*) by Dicer, another RNaseIII-type enzyme. Similar to Drosha, cofactors such as TRBP and PACT (in humans) are necessary for Dicer activity (Lee et al. 2006). The microRNA: microRNA* duplex is in turn loaded into the miRISC complex in which Argonaut (Ago) proteins appear to be the key effector molecules. The strand that becomes the active mature microRNA appears to be dependent upon which has the lowest free energy 5′ end and is retained by the miRISC complex whilst the other strand is usually degraded by an unknown nuclease (Khvorova et al. 2003; Schwarz et al. 2003).

The loaded miRISC is guided by the mature microRNA sequence (19-24 nucleotide) to partially complementary sequences within the 3′UTR (and probably coding sequences and 5′UTR as well) of the target mRNA, leading to inhibition of translation, transcript degradation, or both (Lawrie 2007; Lytle et al. 2007). Although repression of translation without mRNA degradation was originally believed to be the *modus operandi* of animal microRNAs, the situation appears to be more complex than previously thought, as there is now compelling evidence that microRNAs also effect transcriptional levels through de-adenylation and/or degradation (Giraldez et al. 2006) and may even positively affect translation in some instances (Vasudevan et al. 2007). How translational repression occurs remains unclear. It has been suggested that mRNA bound to the microRNA-miRISC complex may be sequestered away from the translational machinery in P-bodies that additionally act in concert with enzymes to remove the 5′-cap hence preventing translation (Liu et al. 2005; Sen & Blau 2005). Alternatively it has been suggested that microRNAs may prevent recognition of the 5′cap by translation factors (Pillai et al. 2005).

4. MicroRNA expression in FL

The following experimental details were taken in part from previously published research (Lawrie, CH et al., 2009). The only other study, as far we are aware, that considers microRNA expression in FL, was by Roehle *et al* which although it included 46 FL samples, only measured levels of 153 microRNA probes (compared with 464 microRNA probes in this study), and did not consider FL transformation (Roehle et al. 2008).

4.1 Materials and methods

4.1.1 Patient material

Formalin-fixed paraffin-embedded (FFPE) biopsy samples from 98 patients were obtained from the Pathology Department of the John Radcliffe Hospital, Oxford, UK. Eighty patients were diagnosed histologically and clinically as having DLBCL; 64 *de novo* (DLBCL-de novo) and 16 transformed cases with previously diagnosed FL (DLBCL-t). Of the 18 cases of FL used in this study, seven subsequently underwent high grade transformation (FL-t) with a median time to transformation of 24 months (range 10-96 months) from initial diagnosis. The remaining 11 FL cases (FL-nt) had no recorded transformation events (median follow-up time 60 months; range 52-132 months). The FL-t and DLBCL-t samples were not paired. All FL cases were grade 1 or 2 at time of original

diagnosis. All samples were collected at time of initial diagnosis (i.e. prior to treatment) with the exception of DLBCL-t cases. Samples had >80% of tumor cells as determined by hematoxylin and eosin staining (not shown). Relevant ethical permission was obtained for the use of all samples.

4.1.2 RNA purification and microarray analysis

Total RNA was purified from four x 20 μm FFPE sections using the Recoverall kit from Ambion (Huntington, UK) in accordance with the manufacturers' instructions. RNA (3 μg) were labeled and hybridized to μRNA microarrays as previously described (Lawrie et al. 2008) using tonsillar material (pooled from twelve healthy individuals) as a common reference in a dye-balanced design.

Image analysis was carried out with BlueFuse software (BlueGnome, Cambridge, UK). Raw image data were global median-normalized within arrays and normalized between arrays using the LIMMA package (Smyth & Speed 2003). The normalized log ratios (average of four replicates per probe) were used for subsequent analysis in Genespring 7.2 (Agilent Technologies, CA, US). ANOVA analysis was used to identify microRNAs differentially expressed between sample types and *P* values were adjusted using the Benjamini-Hodgberg correction method. Differentially expressed genes were tested for their ability to predict sample class using the leave-one-out cross-validation support vector machine (SVM) function in Genespring.

4.2 Results & discussion

4.2.1 MicroRNA expression is distinct between DLBCL and FL

In order to investigate differences in microRNA expression between FL and DLBCL samples, and because *de novo* and transformed DLBCL are indistinguishable histologically, we initially compared expression in all DLBCL cases (n = 80) with that of all FL cases (n = 18). Thirty microRNAs were found to be differentially expressed ($P < 0.05$) (Table 1). Expression values of these microRNAs correctly predicted 95/97 (98%) of cases as DLBCL or FL by SVM, and clustered the cases distinctly (Fig. 2A).

The study by Roehle *et al* identified 10 microRNAs that were differentially expressed between FL and DLBCL cases (Roehle et al. 2008). Only two of these microRNAs (*miR-150* and *miR-135a*) were found to be differentially expressed ($P < 0.05$) in our patient cohort, although another two microRNAs, *miR-92* and *miR-125b*, had *P* values of < 0.1. These 10 microRNAs correctly predicted 74/97 (76%) of cases according to diagnosis.

Roehle's study, however, compared *de novo* cases of DLBCL with FL cases that did not undergo subsequent transformation. Therefore, in order to compare the data directly we used the same sample types (64 DLBCL-de novo and 11 FL-nt cases) to re-analyze the data. This resulted in 26 differentially expressed ($P < 0.05$) microRNAs (Table 2), 14 of which were also present in the previous list (Table 1). These microRNAs correctly predicted 73/75 (97%) of cases in this cohort (c.f. 60/75 (80%) with the 10 microRNA signature (Roehle et al. 2008)) and 92/97 (95%) of cases in the extended cohort. Again, the two sets of samples were found to cluster distinctly using the 26-microRNA signature (Fig. 2B).

microRNA	*P* value	Up	Fold change
hsa-miR-200c	8.20E-08	DLBCL	9.39
hsa-miR-518a	1.23E-03	DLBCL	3.15
hsa-miR-638	8.05E-04	DLBCL	3.09
hsa-miR-205	4.36E-02	DLBCL	2.85
hsa-miR-223	1.42E-02	DLBCL	2.75
hsa-miR-573	2.79E-02	DLBCL	2.35
hsa-miR-135b	3.83E-02	DLBCL	1.63
hsa-miR-133a	8.65E-03	DLBCL	1.38
hsa-miR-135a	3.72E-02	DLBCL	1.38
hsa-miR-451	3.67E-03	DLBCL	1.38
hsa-miR-27b	2.12E-06	DLBCL	1.21
hsa-miR-27a	4.62E-07	DLBCL	1.13
hsa-miR-18b	1.03E-02	DLBCL	0.93
hsa-miR-199b	3.23E-03	DLBCL	0.83
hsa-miR-19a	1.20E-02	DLBCL	0.80
hsa-miR-210	1.10E-02	DLBCL	0.75
hsa-miR-19b	7.60E-04	DLBCL	0.75
hsa-miR-99a	9.00E-05	DLBCL	0.72
hsa-miR-100	1.07E-02	DLBCL	0.51
hsa-miR-361	3.57E-02	FL	0.58
hsa-miR-29c	3.07E-02	FL	0.63
hsa-miR-26a	8.85E-03	FL	0.73
hsa-miR-29b	4.22E-03	FL	0.76
hsa-miR-26b	5.50E-03	FL	1.04
hsa-miR-655	4.76E-02	FL	2.32
hsa-miR-10b	3.10E-02	FL	2.38
hsa-miR-634	1.19E-02	FL	2.41
hsa-miR-593	3.30E-02	FL	2.43
hsa-miR-28	1.47E-02	FL	2.49
hsa-miR-150	1.45E-03	FL	3.37

Table 1. MicroRNAs differentially expressed ($P < 0.05$) between DLBCL (DLBCL-de novo and DLBCL-t) and FL (FL-nt and FL-t) diagnoses.

microRNA	*P* value	Up	Fold change
hsa-miR-200c	4.58E-06	DLBCL	10.03
hsa-miR-638	5.30E-04	DLBCL	3.31
hsa-miR-518a	3.85E-02	DLBCL	2.88
hsa-miR-199a	1.57E-02	DLBCL	2.67
hsa-miR-93	3.74E-02	DLBCL	2.64
hsa-miR-22	1.94E-02	DLBCL	2.46
hsa-miR-34a	3.92E-02	DLBCL	2.39
hsa-miR-362	4.68E-02	DLBCL	2.30
hsa-miR-206	3.93E-02	DLBCL	1.73
hsa-miR-451	3.23E-03	DLBCL	1.49
hsa-miR-636	8.55E-03	DLBCL	1.17
hsa-miR-92	4.05E-02	DLBCL	1.08
hsa-miR-27b	6.85E-04	DLBCL	1.04
hsa-miR-199b	1.15E-04	DLBCL	1.03
hsa-miR-27a	4.03E-04	DLBCL	0.97
hsa-miR-24	2.04E-02	DLBCL	0.75
hsa-miR-106a	1.16E-02	DLBCL	0.73
hsa-miR-20a	2.62E-02	DLBCL	0.67
hsa-miR-19b	4.95E-03	DLBCL	0.64
hsa-miR-99a	9.55E-03	DLBCL	0.56
hsa-miR-18b	3.27E-02	DLBCL	0.54
hsa-miR-100	1.95E-02	DLBCL	0.43
hsa-miR-26b	1.88E-02	FL	1.42
hsa-miR-217	2.84E-02	FL	2.44
hsa-miR-634	9.50E-04	FL	2.54
hsa-miR-150	4.55E-02	FL	3.46

Table 2. MicroRNAs differentially expressed ($P < 0.05$) between *de novo* DLBCL and non-transforming FL cases. Members of the *miR-17-92* cluster (and homologous clusters) are depicted in bold type.

Interestingly, six of the microRNAs that were identified as being up-regulated in DLBCL-*de novo* cases compared to FL-nt are encoded by the *miR-17-92* and/or homologous clusters (average fold-increase of 1.05 (range 0.54-2.64)) (He et al. 2005). The other four microRNAs encoded by these clusters, *miR-17-5p, miR-19a*, *miR-25* and *miR-106b*, had *P* values of 0.067, 0.087, 0.064 and 0.391 respectively. The *miR-17-92* cluster is encoded at the 13q31 locus, a region commonly amplified in lymphomas and ectopic expression of *miR-17-92* greatly accelerated lymphogenesis in a murine model (He et al. 2005). Moreover, direct binding of the c-myc protein up-regulates *miR*-17-92 expression (O'Donnell et al. 2005) and over-expression of c-myc has been demonstrated in the majority (66.6%) of DLBCL cases (Aref et al. 2004), which has also been associated with poorer outcome (Pagnano et al. 2001). An increased level of this cluster in DLBCL compared to FL is consistent with a more aggressive clinical phenotype of DLBCL.

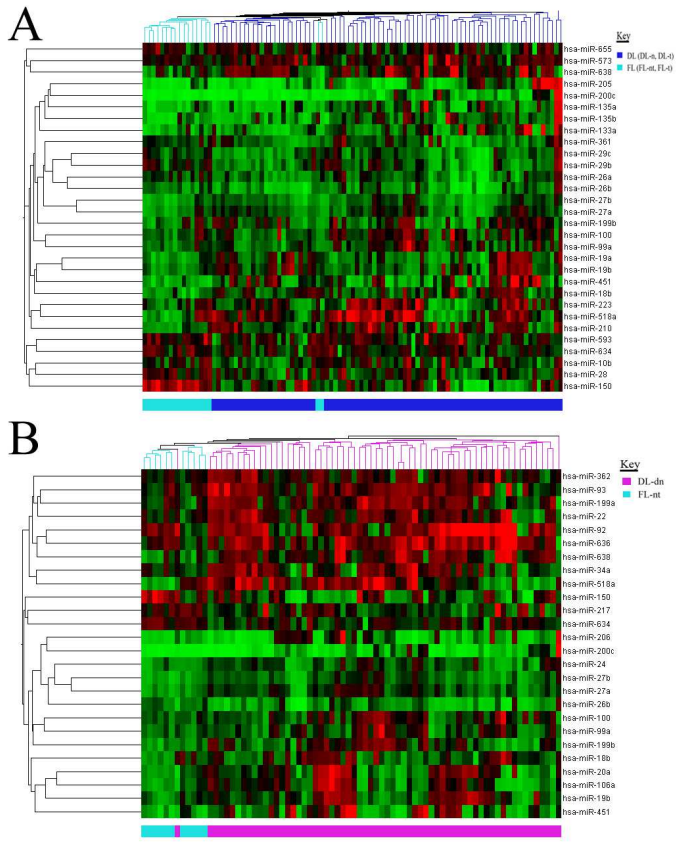

Fig. 2. **Cluster analysis of microRNAs differentially expressed between FL and DLBCL.** (A) All DLBCL cases (n = 80) and FL cases (n = 18). (B) Only *de novo* cases (DLBCL-de novo (n = 64)) and cases of FL that did not undergo transformation (FL-nt (n = 11)). Reproduced from (Lawrie et al. 2009).

4.2.2 Histological transformation of FL is associated with changes in microRNA expression

To investigate whether changes in microRNA expression were associated with transformation we first looked at differences between *de novo* (DLBCL-de novo (n = 64) and transformed (DLBCL-t (n = 16)) cases of DLBCL. Fourteen microRNAs (Table 3) were found to be differentially expressed ($P < 0.05$). These microRNAs correctly predicted transformation status in 73/80 (91%) of samples. Only one of these was up-regulated in DLBCL-t (*miR-491*). Four of the microRNAs down-regulated in DLBCL-t are encoded by the *miR-17-92* cluster suggesting an involvement of the cluster in high grade transformation. An alternative explanation is that because the cluster was also found to be down-regulated in FL compared with DLBCL-de novo (Table 2), the expression pattern of these microRNAs in DLBCL-t cases reflects that of antecedent FL. This latter hypothesis is consistent with gene

expression profile studies that found that DLBCL-t cases were more closely related to FL than DLBCL-de novo cases (Lossos et al. 2002).

microRNA	*P* value	Up	Fold change
hsa-miR-491	2.11E-02	trans	2.54
hsa-miR-27a	3.90E-02	*de novo*	0.47
hsa-miR-19b	3.77E-03	*de novo*	0.60
hsa-miR-25	3.84E-02	*de novo*	0.67
hsa-miR-18a	1.24E-02	*de novo*	0.72
hsa-miR-636	2.73E-02	*de novo*	1.06
hsa-miR-92	1.94E-02	*de novo*	1.14
hsa-miR-621	2.29E-02	*de novo*	1.98
hsa-miR-526c	2.44E-02	*de novo*	2.38
hsa-miR-766	2.75E-02	*de novo*	2.58
hsa-miR-299-5p	4.76E-02	*de novo*	2.61
hsa-miR-380-3p	5.94E-03	*de novo*	2.65
hsa-miR-129	2.98E-02	*de novo*	2.70
hsa-miR-588	9.05E-03	*de novo*	2.80

Table 3. MicroRNAs differentially expressed ($P < 0.05$) between DLBCL-de novo and DLBCL-t cases.

Next we compared FL cases that subsequently underwent high grade transformation (FL-t (n = 7)) with cases that did not (FL-nt (n = 11)). Six microRNAs were differentially expressed ($P < 0.05$) between these two groups (Table 4), whose expression levels correctly predicted 16/18 (89%) of cases.

microRNA	*P* value	Up	
hsa-miR-223	1.43E-03	FL-nt	1.51
hsa-miR-217	5.56E-03	FL-nt	2.56
hsa-miR-222	1.41E-02	FL-t	1.26
hsa-let-7i	2.09E-02	FL-t	2.45
hsa-miR-221	2.34E-02	FL-t	3.14
hsa-let-7b	2.46E-02	FL-t	3.18

Table 4. MicroRNAs differentially expressed ($P < 0.05$) between FL cases that subsequently underwent high grade transformation (FL-t) and those that did not (FL-nt). Median follow-up time 60 months (range 52-132 months).

Let-7b, *let-7i*, *miR-221* and *miR-222* were up-regulated in FL-t whilst *miR-223* and *miR-217* were down-regulated (Fig. 3). Members of the *let-7* family have been shown to target c-myc

expression in Burkitt lymphoma (Sampson et al. 2007) and decreased c-myc expression has been associated with high grade transformation of FL to DLBCL (Lossos et al. 2002). Interestingly, up-regulated microRNAs *miR-221* and *miR-222* target the tumor suppressor molecule p27(Kip1) (le Sage et al. 2007) whilst down-regulated *miR-223* has been shown to target Stathmin, a known oncogene (Alli et al. 2007). Although the number of cases in this analysis was small, and requires further validation, these data open up the exciting possibility that microRNA expression could be used to predict FL patients at risk of transformation that could benefit from an up-front aggressive therapy regimen.

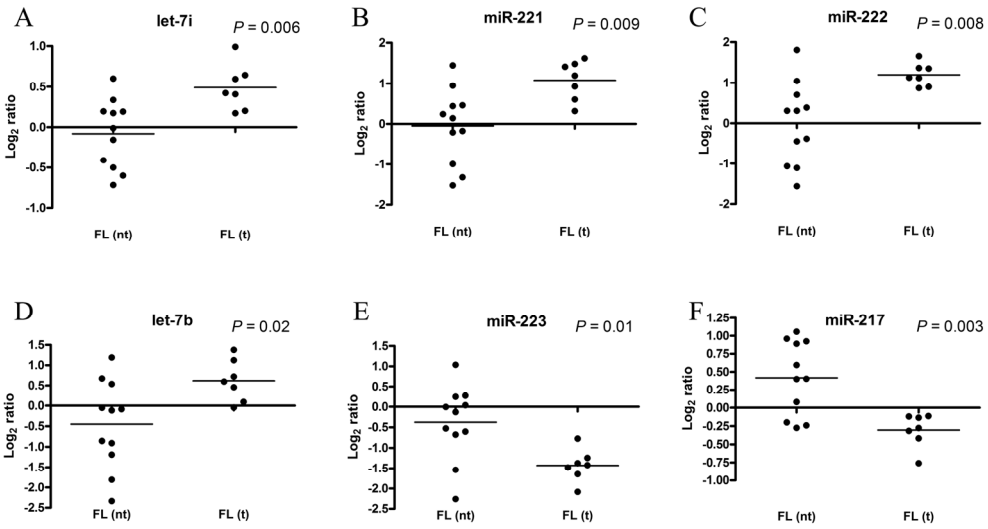

Fig. 3. **Expression levels of microRNAs differentially expressed between FL-t and FL-nt cases.** *P* values were calculated by independent *t*-test. Reproduced from (Lawrie et al. 2009).

5. Conclusion

In this chapter we have discussed some of the clinico-scientific issues pertaining to follicular lymphoma and the role that microRNAs may play in both its pathogenesis and in particular histological high grade transformation. As is outlined in this article there are in fact only two pieces of research published to date that have investigated microRNA expression in FL, and hence some caution should be applied when drawing conclusions about the role/potential of specific microRNAs in this disease, as clearly much more research is required. Nonetheless, these studies do present some interesting insights and offer the tantalizing possibility that microRNAs may deliver novel biomarkers that can identify FL patients at risk of transformation where other molecular techniques have failed.

6. Acknowledgments

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7. References

Alli, E, Yang, JM & Hait, WN (2007). Silencing of stathmin induces tumor-suppressor function in breast cancer cell lines harboring mutant p53. *Oncogene*, 26(7), pp.1003-1012.

Altuvia, Y, Landgraf, P, Lithwick, G, Elefant, N, Pfeffer, S, Aravin, A, Brownstein, MJ, Tuschl, T & Margalit, H (2005). Clustering and conservation patterns of human microRNAs. *Nucleic Acids Res*, 33(8), pp.2697-2706.

Anderson, JR, Armitage, JO & Weisenburger, DD (1998). Epidemiology of the non-Hodgkin's lymphomas: distributions of the major subtypes differ by geographic locations. Non-Hodgkin's Lymphoma Classification Project. *Ann Oncol*, 9(7), pp.717-720.

Aref, S, Mabed, M, Zalata, K, Sakrana, M & El Askalany, H (2004). The interplay between c-Myc oncogene expression and circulating vascular endothelial growth factor (sVEGF), its antagonist receptor, soluble Flt-1 in diffuse large B cell lymphoma (DLBCL): relationship to patient outcome. *Leuk Lymphoma*, 45(3), pp.499-506.

Berglund, M, Enblad, G, Thunberg, U, Amini, RM, Sundstrom, C, Roos, G, Erlanson, M, Rosenquist, R, Larsson, C & Lagercrantz, S (2007). Genomic imbalances during transformation from follicular lymphoma to diffuse large B-cell lymphoma. *Mod Pathol*, 20(1), pp.63-75.

Borchert, GM, Lanier, W & Davidson, BL (2006). RNA polymerase III transcribes human microRNAs. *Nat Struct Mol Biol*, 13(12), pp.1097-1101.

Calin, GA, Sevignani, C, Dumitru, CD, Hyslop, T, Noch, E, Yendamuri, S, Shimizu, M, Rattan, S, Bullrich, F, Negrini, M & Croce, CM (2004). Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proc Natl Acad Sci U S A*, 101(9), pp.2999-3004.

Davies, AJ, Rosenwald, A, Wright, G, Lee, A, Last, KW, Weisenburger, DD, Chan, WC, Delabie, J, Braziel, RM, Campo, E, Gascoyne, RD, Jaffe, ES, Muller-Hermelink, K, Ott, G, Calaminici, M, Norton, AJ, Goff, LK, Fitzgibbon, J, Staudt, LM & Andrew Lister, T (2007). Transformation of follicular lymphoma to diffuse large B-cell lymphoma proceeds by distinct oncogenic mechanisms. *Br J Haematol*, 136(2), pp.286-293.

de Vos, S, Hofmann, WK, Grogan, TM, Krug, U, Schrage, M, Miller, TP, Braun, JG, Wachsman, W, Koeffler, HP & Said, JW (2003). Gene expression profile of serial samples of transformed B-cell lymphomas. *Lab Invest*, 83(2), pp.271-285.

Elenitoba-Johnson, KS, Jenson, SD, Abbott, RT, Palais, RA, Bohling, SD, Lin, Z, Tripp, S, Shami, PJ, Wang, LY, Coupland, RW, Buckstein, R, Perez-Ordonez, B, Perkins, SL, Dube, ID & Lim, MS (2003). Involvement of multiple signaling pathways in follicular lymphoma transformation: p38-mitogen-activated protein kinase as a target for therapy. *Proc Natl Acad Sci U S A*, 100(12), pp.7259-7264.

Friedman, RC, Farh, KK, Burge, CB & Bartel, DP (2009). Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res*, 19(1), pp.92-105.

Giraldez, AJ, Mishima, Y, Rihel, J, Grocock, RJ, Van Dongen, S, Inoue, K, Enright, AJ & Schier, AF (2006). Zebrafish MiR-430 promotes deadenylation and clearance of maternal mRNAs. *Science*, 312(5770), pp.75-79.

Glas, AM, Knoops, L, Delahaye, L, Kersten, MJ, Kibbelaar, RE, Wessels, LA, van Laar, R, van Krieken, JH, Baars, JW, Raemaekers, J, Kluin, PM, van't Veer, LJ & de Jong, D (2007). Gene-expression and immunohistochemical study of specific T-cell subsets and accessory cell types in the transformation and prognosis of follicular lymphoma. *J Clin Oncol*, 25(4), pp.390-398.

Griffiths-Jones, S, Grocock, RJ, van Dongen, S, Bateman, A & Enright, AJ (2006). miRBase: microRNA sequences, targets and gene nomenclature. *Nucleic Acids Res*, 34(Database issue), pp.D140-144.

He, L, Thomson, JM, Hemann, MT, Hernando-Monge, E, Mu, D, Goodson, S, Powers, S, Cordon-Cardo, C, Lowe, SW, Hannon, GJ & Hammond, SM (2005). A microRNA polycistron as a potential human oncogene. *Nature*, 435(7043), pp.828-833.

Hertel, J, Lindemeyer, M, Missal, K, Fried, C, Tanzer, A, Flamm, C, Hofacker, IL & Stadler, PF (2006). The expansion of the metazoan microRNA repertoire. *BMC Genomics*, 7, pp.25.

Iorio, MV & Croce, CM (2009). MicroRNAs in cancer: small molecules with a huge impact. *J Clin Oncol*, 27(34), pp.5848-5856.

Iqbal, J, Sanger, WG, Horsman, DE, Rosenwald, A, Pickering, DL, Dave, B, Dave, S, Xiao, L, Cao, K, Zhu, Q, Sherman, S, Hans, CP, Weisenburger, DD, Greiner, TC, Gascoyne, RD, Ott, G, Muller-Hermelink, HK, Delabie, J, Braziel, RM, Jaffe, ES, Campo, E, Lynch, JC, Connors, JM, Vose, JM, Armitage, JO, Grogan, TM, Staudt, LM & Chan, WC (2004). BCL2 translocation defines a unique tumor subset within the germinal center B-cell-like diffuse large B-cell lymphoma. *Am J Pathol*, 165(1), pp.159-166.

Jaffe, ES, Harris, NL, Stein, H & Vardiman, J, Eds. (2001). World Health Organisation (WHO) Classification of Tumours-Pathology & Genetics-Tumours of Haematopoietic and Lymphoid Tissues. Lyon, IARC Press (International Agency for Research on Cancer).

Khvorova, A, Reynolds, A & Jayasena, SD (2003). Functional siRNAs and miRNAs exhibit strand bias. *Cell*, 115(2), pp.209-216.

Kim, VN (2005). MicroRNA biogenesis: coordinated cropping and dicing. *Nat Rev Mol Cell Biol*, 6(5), pp.376-385.

Lawrie, CH (2007). MicroRNAs and haematology: small molecules, big function. *Br J Haematol*, 137(6), pp.503-512.

Lawrie, CH (2008). microRNA expression in lymphoid malignancies: new hope for diagnosis and therapy? *J Cell Mol Med*, 12(5A), pp.1432-1444.

Lawrie, CH, Chi, J, Taylor, S, Tramonti, D, Ballabio, E, Palazzo, S, Saunders, NJ, Pezzella, F, Boultwood, J, Wainscoat, JS & Hatton, CS (2009). Expression of microRNAs in diffuse large B cell lymphoma is associated with immunophenotype, survival

and transformation from follicular lymphoma. *J Cell Mol Med*, 13(7), pp.1248-1260.

Lawrie, CH, Saunders, NJ, Soneji, S, Palazzo, S, Dunlop, HM, Cooper, CD, Brown, PJ, Troussard, X, Mossafa, H, Enver, T, Pezzella, F, Boultwood, J, Wainscoat, JS & Hatton, CS (2008). MicroRNA expression in lymphocyte development and malignancy. *Leukemia*, 22(7), pp.1440-1446.

le Sage, C, Nagel, R, Egan, DA, Schrier, M, Mesman, E, Mangiola, A, Anile, C, Maira, G, Mercatelli, N, Ciafre, SA, Farace, MG & Agami, R (2007). Regulation of the p27(Kip1) tumor suppressor by miR-221 and miR-222 promotes cancer cell proliferation. *Embo J*, 26(15), pp.3699-3708.

Lee, RC & Ambros, V (2001). An extensive class of small RNAs in Caenorhabditis elegans. *Science*, 294(5543), pp.862-864.

Lee, RC, Feinbaum, RL & Ambros, V (1993). The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell*, 75(5), pp.843-854.

Lee, Y, Hur, I, Park, SY, Kim, YK, Suh, MR & Kim, VN (2006). The role of PACT in the RNA silencing pathway. *Embo J*, 25(3), pp.522-532.

Liu, J, Valencia-Sanchez, MA, Hannon, GJ & Parker, R (2005). MicroRNA-dependent localization of targeted mRNAs to mammalian P-bodies. *Nat Cell Biol*, 7(7), pp.719-723.

Lossos, IS, Alizadeh, AA, Diehn, M, Warnke, R, Thorstenson, Y, Oefner, PJ, Brown, PO, Botstein, D & Levy, R (2002). Transformation of follicular lymphoma to diffuse large-cell lymphoma: alternative patterns with increased or decreased expression of c-myc and its regulated genes. *Proc Natl Acad Sci U S A*, 99(13), pp.8886-8891.

Lytle, JR, Yario, TA & Steitz, JA (2007). Target mRNAs are repressed as efficiently by microRNA-binding sites in the 5' UTR as in the 3' UTR. *Proc Natl Acad Sci U S A*, 104(23), pp.9667-9672.

McDonnell, TJ & Korsmeyer, SJ (1991). Progression from lymphoid hyperplasia to high-grade malignant lymphoma in mice transgenic for the t(14; 18). *Nature*, 349(6306), pp.254-256.

Montoto, S, Davies, AJ, Matthews, J, Calaminici, M, Norton, AJ, Amess, J, Vinnicombe, S, Waters, R, Rohatiner, AZ & Lister, TA (2007). Risk and clinical implications of transformation of follicular lymphoma to diffuse large B-cell lymphoma. *J Clin Oncol*, 25(17), pp.2426-2433.

O'Donnell, KA, Wentzel, EA, Zeller, KI, Dang, CV & Mendell, JT (2005). c-Myc-regulated microRNAs modulate E2F1 expression. *Nature*, 435(7043), pp.839-843.

Pagnano, KB, Vassallo, J, Lorand-Metze, I, Costa, FF & Saad, ST (2001). p53, Mdm2, and c-Myc overexpression is associated with a poor prognosis in aggressive non-Hodgkin's lymphomas. *Am J Hematol*, 67(2), pp.84-92.

Pasquinelli, AE, Reinhart, BJ, Slack, F, Martindale, MQ, Kuroda, MI, Maller, B, Hayward, DC, Ball, EE, Degnan, B, Muller, P, Spring, J, Srinivasan, A, Fishman, M, Finnerty, J, Corbo, J, Levine, M, Leahy, P, Davidson, E & Ruvkun, G (2000). Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. *Nature*, 408(6808), pp.86-89.

Pillai, RS, Bhattacharyya, SN, Artus, CG, Zoller, T, Cougot, N, Basyuk, E, Bertrand, E & Filipowicz, W (2005). Inhibition of translational initiation by Let-7 MicroRNA in human cells. *Science*, 309(5740), pp.1573-1576.

Reinhart, BJ, Slack, FJ, Basson, M, Pasquinelli, AE, Bettinger, JC, Rougvie, AE, Horvitz, HR & Ruvkun, G (2000). The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans. *Nature*, 403(6772), pp.901-906.

Rodriguez, A, Griffiths-Jones, S, Ashurst, JL & Bradley, A (2004). Identification of mammalian microRNA host genes and transcription units. *Genome Res*, 14(10A), pp.1902-1910.

Roehle, A, Hoefig, KP, Repsilber, D, Thorns, C, Ziepert, M, Wesche, KO, Thiere, M, Loeffler, M, Klapper, W, Pfreundschuh, M, Matolcsy, A, Bernd, HW, Reiniger, L, Merz, H & Feller, AC (2008). MicroRNA signatures characterize diffuse large B-cell lymphomas and follicular lymphomas. *Br J Haematol*, 142(5), pp.732-744.

Rossi, D, Berra, E, Cerri, M, Deambrogi, C, Barbieri, C, Franceschetti, S, Lunghi, M, Conconi, A, Paulli, M, Matolcsy, A, Pasqualucci, L, Capello, D & Gaidano, G (2006). Aberrant somatic hypermutation in transformation of follicular lymphoma and chronic lymphocytic leukemia to diffuse large B-cell lymphoma. *Haematologica*, 91(10), pp.1405-1409.

Roulland, S, Navarro, JM, Grenot, P, Milili, M, Agopian, J, Montpellier, B, Gauduchon, P, Lebailly, P, Schiff, C & Nadel, B (2006). Follicular lymphoma-like B cells in healthy individuals: a novel intermediate step in early lymphomagenesis. *J Exp Med*, 203(11), pp.2425-2431.

Sampson, VB, Rong, NH, Han, J, Yang, Q, Aris, V, Soteropoulos, P, Petrelli, NJ, Dunn, SP & Krueger, LJ (2007). MicroRNA let-7a down-regulates MYC and reverts MYC-induced growth in Burkitt lymphoma cells. *Cancer Res*, 67(20), pp.9762-9770.

Schwarz, DS, Hutvagner, G, Du, T, Xu, Z, Aronin, N & Zamore, PD (2003). Asymmetry in the assembly of the RNAi enzyme complex. *Cell*, 115(2), pp.199-208.

SEER (2005). Cancer Statistics Review, 1975-2002. *National Cancer Institute*.

Sen, GL & Blau, HM (2005). Argonaute 2/RISC resides in sites of mammalian mRNA decay known as cytoplasmic bodies. *Nat Cell Biol*, 7(6), pp.633-636.

Smyth, GK & Speed, T (2003). Normalization of cDNA microarray data. *Methods*, 31(4), pp.265-273.

Strasser, A, Harris, AW & Cory, S (1993). E mu-bcl-2 transgene facilitates spontaneous transformation of early pre-B and immunoglobulin-secreting cells but not T cells. *Oncogene*, 8(1), pp.1-9.

Tsujimoto, Y, Cossman, J, Jaffe, E & Croce, CM (1985). Involvement of the bcl-2 gene in human follicular lymphoma. *Science*, 228(4706), pp.1440-1443.

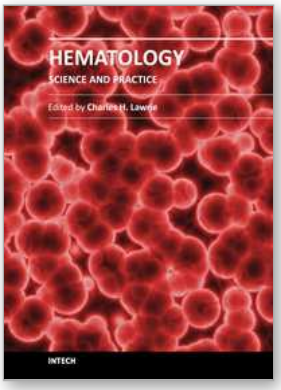
Vasudevan, S, Tong, Y & Steitz, JA (2007). Switching from repression to activation: microRNAs can up-regulate translation. *Science*, 318(5858), pp.1931-1934.

Wightman, B, Ha, I & Ruvkun, G (1993). Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in C. elegans. *Cell*, 75(5), pp.855-862.

Wrench, D, Montoto, S & Fitzgibbon, J (2010). Molecular signatures in the diagnosis and management of follicular lymphoma. *Current Opinion in Hematology*, 17(4), pp.333-340.

Yeom, KH, Lee, Y, Han, J, Suh, MR & Kim, VN (2006). Characterization of DGCR8/Pasha, the essential cofactor for Drosha in primary miRNA processing. *Nucleic Acids Res*, 34(16), pp.4622-4629.

Zeng, Y (2006). Principles of micro-RNA production and maturation. *Oncogene*, 25(46), pp.6156-6162.



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Hematology encompasses the physiology and pathology of blood and of the blood-forming organs. In common with other areas of medicine, the pace of change in hematology has been breathtaking over recent years. There are now many treatment options available to the modern hematologist and, happily, a greatly improved outlook for the vast majority of patients with blood disorders and malignancies. Improvements in the clinic reflect, and in many respects are driven by, advances in our scientific understanding of hematological processes under both normal and disease conditions. Hematology - Science and Practice consists of a selection of essays which aim to inform both specialist and non-specialist readers about some of the latest advances in hematology, in both laboratory and clinic.

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