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Gene expression profiling for the diagnosis and prognosis of acute myeloid leukaemia

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1. ABSTRACT

Acute myeloid leukaemia (AML) is a heterogeneous disease covering a range of morphological lineages and differentiation stages, but also has number of recurrent chromosomal abnormalities and mutations associated with prognosis. Because of the defined molecular and cytogenetic features, AML has been a focus of gene expression profiling studies and identified differentially expressed genes in the different diagnostic and cytogenetic sub-groups. These studies have lead to the development of expression based classifications and diagnostic chips with further studies aiding prognosis and therapeutic choices.

2. INTRODUCTION

Acute myeloid leukemia (AML) is a clinically and molecular heterogeneous set of hematopoietic disorders (1) which account for 77% of acute leukemia (2). The overall incidence of AML is approximately 4 in 100,000 in the United States and United Kingdom, and increases with age from 1 in 100,000 in people younger than 35 years old at diagnosis to 15 in 100,000 in those older than 75 years.

AML is characterized by the infiltration of bone marrow by abnormal hematopoietic progenitors that disrupts normal production of erythroid, myeloid, and/or megakaryocytic cells. The deregulated proliferation and impaired differentiation result in an increased survival advantage for the leukemia cell (3-5) which expand in number eventually dominating the normal hematopoiesis.

AML has been classified using morphological assessment of lineage, commitment, and differentiation, the French–American–British (FAB) system (6), however, the World Health Organization (WHO) has introduced a new classification (7) which places the most common and well-defined recurring cytogenetic abnormalities into separate major groups with further defined classified subgroups. Many types of AML are associated with balanced reciprocal translocations involving two transcription factors. These transcription factors are frequently conserved in evolution and are important both in mammalian development as well as in normal hematopoiesis. Transcription factors involved in human leukemia include core binding factor (CBF), retinoic acid receptor α (RARα), homeobox (HOX) family members,

and members of the ETS family of transcription factors. Some of these chromosomal abnormalities correlate with specific FAB subtypes, for instance, the translocation t (8;21) is present in approximately 40% of the FAB-M2 leukemia (8). Translocations involving the retinoic receptor α (RARα) on chromosome 17 in APL (APML) FAB-M3 OR M3Vt (15;17) is present in 98% of cases (9) and inv (16) or t (16;16) involved in the FAB-M4-Eo cases (10;11).

3. CYTOGENETIC AND MOLECULAR ABNORMALITIES

The cytogenetic characteristics have been used to classify patients into three prognostic subgroups or risk groups: those with favorable or good risk, intermediate or standard risk, and unfavorable, adverse, or poor risk disease. The favorable risk group (12) includes patients with either t (15;17), t (8;21) and inv (16) or t (16;16) cytogenetic translocations. The poor prognosis group includes cytogenetic abnormalities such as deletion of chromosomes 5 or 7 (or the long-arms of the chromosomes) abnormalities in the long-arm of chromosome 3 and AML with complex karyotype (three or more cytogenetic aberrations) (13;14). Cytogenetic mutations involving the 11q23 region are also considered a marker for poor prognosis. Patients presenting with AML with other type of cytogenetic abnormalities or without any apparent cytogenetic abnormalities (i.e., an apparently normal karyotype) are considered to have an intermediate risk; (1;14).

3.1. t (15;17)

APL (FAB subtype M3) accounts for approximately 10% of all AML cases (15) and is characterized by translocations involving the *RARα* gene located at 17q12. In the majority of the cases, the partner chromosome in the translocation is chromosome 15 resulting in the fusion of large parts of RARα to most of PML coding sequence, generating the PML-RARα fusion protein (16;17). Rarer cases of APL show translocations involving t (11;17) (q23;q12), t (5;17) (q35q12), t (17;17) (q11;q12), or t (11;17) (q13;q12). These result in fusion proteins which also contain RARα but with different partner genes: PLZF (promyelocytic leukemia zinc finger), NPM (nucleophosmin), Stat5b or NuMA (18-24).

3.2. Core binding factor abnormalities

Other balanced abnormalities frequently seen are the t (8;21) and inv (16) or t (16;16) translocations which disrupt the "core binding factor (CBF) complex," and this type of translocation is observed in approximately 20% of AML cases, particularly in those patients less than 60 years of age at diagnosis. (25). The CBF consists of two subunits: AML1 (also referred to as RUNX1, CBPα2, and PEBP2αB) and is located on chromosome 21q22 and CBFβ, which is encoded by a gene on 16q22. The two subunits form a heterodimer transcription activator, although only the AML1 subunit contains DNA binding ability.

3.2.1. t (8;21)

The most frequent translocation is the t (8;21) translocation, found in 10%–15% of adult patients with this disease (25) and results in the fusion protein AML1-ETO. The C-terminus of the transcriptional activator AML1 is replaced by the transcriptional repressor ETO, resulting in the fusion protein AML1-ETO (RUNX1-RUNXT1) (26). The ETO protein binds to co-repressors (e.g., NcoR, SMRT, and mSin3) and histone deacetylases (HDACs) (27;28) The HDACs within the complex alters the histone acetylation status which affects DNA conformation and results in repression of AML1 target genes including include granulocyte–macrophage colony-stimulating factor (GM-CSF) (29), for interleukin-3 (IL-3 and NP3 (30).

AML1-ETO influences various other transcription factors including MEF, c/EBPα, AP-1, and Pu.1 (31-33). c/EBPα is a major regulator of early granulocytic differentiation and AML-ETO physically interacts with c/EBPα and downregulates it at the transcriptional level (32), which has an important influence on granulocytic differentiation. AML1-ETO also directly inhibits the expression of $p14^{ARF}$ (34), and inducing p53-dependent proliferation arrest and apoptosis (35). In addition, AML1-ETO also up-regulates Bcl2 (36) whilst the down-regulation of c/EBPα by AML1-ETO resulting in the release of cell cycle control and enhanced cellular proliferation.

3.2.2. inv (16) or t (16;16)

Other CBF leukemia associated translocations in AML are inv (16) and t (16;16) in which CBFβ is fused to MYH11, also referred to as the smooth muscle myosin heavy chain (*SMMHC*) gene (37), generating a CBFβ/SMMHC fusion protein. The fusion protein binds to AML1, and through DNA contact acts as a repressor of AML1 function (38). In addition, the SMMHC part of the fusion protein contains a repressor domain that directly represses AML1 activity when bound to transcriptional active sites (38).

Recent studies on shared target genes of the fusion proteins AML1-ETO, PML-RARα, and PLZF-RARα in U937 cells reported that several of the genes regulated by all three fusion proteins are associated with Wnt-signaling (39). The Wnt-signaling pathway involves both known tumor suppressors or proto-oncogenes in the pathogenesis of epithelial cancers and have been implicated in the self-renewal and proliferation of hematopoietic progenitor cells (40). A positive transcriptional regulator induced by this pathway is γ-catenin (plakoglobin), which was strongly induced by all three fusion proteins. The enhanced transcriptional activity of γ-catenin leads to induction of Wnt target genes resulting in enhanced self-renewal and proliferative capacity of myeloid progenitor cells.

3.3. MLL – 11q23 abnormalities

A subgroup of AML patients have translocations involving the MLL gene located on chromosome 11q23. MLL has histone methyltransferase activity and assembles in very large multi-protein complexes composed of more than 29 proteins (41) whilst the DNA binding domain of MLL contains three AT-hooks that allow regulatory transcription factors to bind to DNA (42). MLL is a very promicious gene and is involved in translocations with

numerous partner chromosomes. More than 60 cytogenetic translocations involving the region 11q23 have been described, with at least 30 of the fusion partners of MLL defined (43); common translocations involving chromosome 11q23 result from a fusion chromosomes 4, 9, or 19

All fusion proteins contain the N-terminal part including the DNA binding domain and the repressor domain of MLL fused to the C-terminal part of the translocation partner. Most of the known MLL fusion partners, for example, ENL, AF9, and AF4 contain transcriptional activation domains. MLL fusion proteins do regulate the expression of *HOX* genes, and this is partially responsible for their ability to immortalize myeloid progenitor cells. MLL regulates HOX genes expression through direct binding to promoter sequences, and HOXA9 is highly expressed in MLL leukemia (44).

In addition to chromosomal translocations, a different type of rearrangement of the *MLL* gene has been described in AML. PTDs result in a duplication of a portion of the gene and have been observed in 3%–10% of adult AML, often associated with trisomy 11 (45;46), but more frequently in 10% of cases without normal cytogenetics. The incidence of MLL PTD in FLT3-ITD positive patients is significantly higher than in FLT3-ITD negative patients (46). This may be due to the fact that FLT3 and MLL loci are susceptible to similar agents and mechanisms of DNA damage and links a mechanism leading to enhanced proliferation with a mechanism resulting in blocked differentiation.

3.4.Other associated mutations or abnormalities

3.4.1. NPM

The *NPM1* gene can be disrupted by reciprocal chromosomal retain the N-terminus of NPM. However, heterozygous mutations of the *NPM1* gene, involving the C-terminus at exon 12, have been found, uniquely to AML, and more than 26 different *NPM1* mutations have been identified which are characterized by simple 1- or 2-tetranucleotide insertions, a 4-base pair (bp) or 5-bp deletion combined with a 9-bp insertion, or a 9-bp deletion combined with a 14-bp insertion (47-49). The most common mutation, known as type A, is an insertion of a TCTG tetranucleotide at positions 956 through 959. Despite the genetic heterogeneity, all *NPM1* gene mutations result in a distinct sequence in the NPM protein at the C-terminus. AML patients with a normal karyotype have a complete remission rate significantly higher than that of patients with wild-type *NPM1* AML and most studies have shown a statistical trend toward favorable outcome in event-free survival and overall survival. Further analyses in the context of other molecular aberrations have shown that patients with *NPM1*-mutated AML who have normal karyotype and lack *FLT3*-ITD (representing about 30% of all AML cases) had a better prognosis (50). *NPM1* gene mutations are frequently associated with *FLT3* mutation of ITD type suggesting a synergistic link between these mutations as patients with dual mutations have less favorable prognosis than patients without *NPM1*/*FLT3* gene mutations (48;49;51).

3.4.2. FLT3

Activating mutations within the FLT3 gene have been identified as both possible progression factors and drug targets in AML. FLT3 (Fms-like tyrosine kinase 3, also designated Flk-2 or STK-1) is a member of the class III receptor tyrosine kinases (RTK) and shares high homology with the other family members, including the PDGF (platelet-derived growth factor) receptors, c-FMS/M-CSF (macrophage colony stimulating factor) receptor, and Kit (receptor for SCF, the stem cell factor). Common features of class III RTK include an extra-cellular domain consisting of five to seven immunoglobulin-like subdomains and a split kinase domain (52). The gene encoding FLT3 is located on chromosome 13q12 (53). The gene contained 24 exons, with exons 14 and 15 encoding the juxta-membrane region of the receptor and exon 20 encoding the tyrosine kinase domain (TKD) (54).

In AML, FLT3 is highly expressed in 60%–92% of the cases (55), and this is at a higher level compared to normal hematopoietic progenitors. FLT3 has been shown to have in-frame insertions of several nucleotides in the mRNA sequence encoding the juxtamembrane domain of the receptor in AML patients (56). These insertions are internal tandem duplications (ITDs) of varying lengths and result in the repetition of between 3 and 50 amino acids in the juxtamembrane region of the FLT3 receptor. Most patients also express the wild-type allele. The prevalence of FLT3-ITD in AML patients have indicated an incidence of between 20% and 30% (57-60). The frequency of in FAB subgroups of AML differs with a relatively high frequency in the APL (58). Highest prevalence of FLT3-ITD mutations was seen in cases with normal karyotype but occur rarely in CBF (inv (16) or t (16;16) or t (8;21)) leukemia (58), where conversely Kit mutations are seen with high frequency.

In APL (M3 or M3v) , FLT3-ITD mutations are associated with high white blood counts, a high percentage of bone marrow blasts, and high levels of LDH at diagnosis (59;61-63). The presence of FLT3-ITD has been associated with an unfavorable prognosis in all FAB subgroups as a result of reduced disease free and overall survival (57;61;62). ITD mutations have a higher incidence with increasing age (64).

The ITD-mutated alleles and the wild-type allele are usually transcribed so that both are co-expressed in AML blasts. However, the wild-type allele is frequently lost in some leukemia clones, resulting in loss of expression of the wild-type mRNA which has been shown to be due to homologous recombination and subsequent loss of heterozygosity as a form of uniparental disomy (62;65). In some patients, FLT3-ITD mutations were not detected at initial diagnosis but were found to be present at relapse, while in others they were present at first diagnosis and undetectable at relapse (59;66). Therefore the use of FLT3-mutations for minimal residual disease detected may be limited.

A different type of FLT3 mutation has also been identified within the activation loop, or the TKD, in

approximately 7% of AML patients (60;67). The activation loop is a highly conserved protein structure in the membrane-distal portion of the kinase domain of several receptor tyrosine kinases. This type of mutation usually affects the codon 835 (D835) where the point mutations alter the amino acid at that codon. Few studies have correlated TKD mutations with prognosis (68). Numerous other point mutations within the TKD have been observed near codon 835, for example, at codons 836, 839, or 840 (60;69).

3.4.3. HOX genes

HOX gene-family members are also involved in AML-associated chromosomal translocations including t (7;11) and t (2;11) which generate the fusion proteins NUP98/HoxA9 and NUP98/HOXD13. NUP98 encodes for a component of the nuclear core complex. The fusion protein consists of the homeodomain of HOXA9 and the phenylalanine–glycine repeats from NUP98 (70-72), which contains an interaction domain with the transcriptional activator CBP/p300. Over-expression of the fusion protein induces a myeloproliferative disease, followed by the occurrence of AML in transplanted mice (73).

3.4.4. KIT

Activating mutations of Kit, also a class III RTK, occur in subgroups of AML affecting two regions of the receptor: the juxta-membrane region and also in residues within in the activation loop (74). These mutations are very frequent in the AML patients with a translocation affecting the CBF: t (8;21) and inv (16) chromosomal translocations. In one study, 24% of patients with inv (16) or t (16;16) had an exon 8 mutation, but in only 2% in patients with t (8;21). However, mutations of residue 816 in exon 17 occurred with similar frequencies (7.9% and 10.6%, respectively) (75). This indicates that different mutations in tyrosine kinase receptors may occur in the different types of AML, and they are probably dependent on, and highly specificfor, accompanying molecular alterations in transcription factors.

3.4.5. RAS

Activating Ras mutations have been described in many reports. These are usually at codon 12 and 13 of N-Ras (76), also codon 61 of N-Ras but rarely in K-Ras, and very rarely in H-Ras. Overall, the incidence of N-Ras mutations is between 10% and 27% (77). The presence of a Ras mutations has been reported to correlate with low blast counts and an improved survival of patients, Ras mutations MDS patients correlated with an increased risk of progression to AML (77;78) this would suggest that Ras mutations are not an initiating event but may be important for disease progression.

Pathway analysis via gene expression profiling of AML samples might reveal activated signaling pathways that could be traced back to identify candidates that provide key signals important for leukemia transformation. Since more and more microarray data of AML bone marrow are being added to the databases, such approaches become increasingly feasible.

4. DISEASE CLASSIFICATION BY MICROARRAY

The first approach to disease classification, based on microarrays, was described in 1999 (79) and used an unsupervised, class discovery approach, identifying previously unrecognized subtypes, to uncover the distinction between AML and acute lymphoblastic leukemia (ALL). The authors speculated that (79) a larger sample would enable finer subclassifications to be identified that would correspond to existing subclassifications for AML or define new groupings. This concept sparked numerous publications trying to identify gene expression profiles or signatures that could distinguish between the major subclasses within AML.

4.1. Analysis of cytogenetic sub-groups

Most of the early microarray studies were done on small numbers (>60 patients) with cytogenetically defined groups of AML samples; these groupings included t (8;21); t (15;17), inv (16) or t (16;16) , 11q23 abnormalities, or normal cytogenetics (80-86). For example, oligonucleotide arrays were used to examine 37 samples from distinct cytogenetic subtypes of AML: t (8;21), inv (16) or t (16;16) , and t (15;17), which are associated with four morphological subgroups (M2, M4Eo, M3, and M3v). Significance of microarray analysis (SAM) (87), and other analyses were used to identify genes whose profiles accurately discriminated between the cytogenetic classes. Furthermore, they showed that only 13 genes were needed to separate the AML samples into their distinctive subgroups, indicating that AML-specific cytogenetic aberrations can be correlated with corresponding gene expression profiles.

A similar study on 28 AML patients used statistical group comparison to identify 145 genes and then hierarchical clustering to separate major cytogenetic classes: t (8;21), t (15;17), inv (16) or t (16;16) , 11q23, and normal karyotype (81). The study identified that members of the class I HOX A and B gene families showed a distinct up-regulation within the normal karyotype group, which may imply a common genetic lesion within these pseudo-normal cytogenetics.

Patients with normal cytogenetics constitute the single largest group in AML; however, trisomy 8 is the most common numerical aberration observed as either a sole abnormality or part of more complex cytogenetics. Patients with normal cytogenetic versus patients with trisomy 8 as the sole abnormality (85) could be divided into two genetically very similar populations with 29 genes up-regulated and located on chromosome 8.

A more focused approach compared patients with APL with the t (15;17) translocation and patients with AML FAB group M1 (AML without maturation characterized by morphologically and phenotypically immature AML blasts and no recurrent chromosomal abnormalities) (82). A multivariate sigma-classifier algorithm could distinguish FAB-M3 or M3Vwith t (15;17) from the AML M1 subgroup, although unsurprisingly, this study showed that two morphologically defined subgroups

(FAB-M1 and FAB-M3) had distinct gene expression signatures. Patients with variants of APL (M3 or M3V (abnormal pro-myelocytes with heavy granulation and bundles of Auer rods) or M3v (non- or hypogranular cytoplasm and a bi-lobed nucleus)) could be separated from other AML with defined cytogenetic abnormalities or those with a normal cytogenetic (86). Further supervised pair-wise comparison showed discrimination between M3 and M3v, based on gene signatures, with a median classification accuracy of 90%.

4.2. Array based classifications

A more recent study (88) used cDNA arrays on 116 adults with AML (including 45 with a normal cytogenetic) and identified 6283 genes that had variable expression. They found that, in common with previous studies, samples with t (15;17) had a highly correlated pattern of expression. However, they also identified gene signatures for patients with t (8;21), inv (16) or t (16;16) or normal cytogenetics, although each group was further subdivided into separate clusters. Samples with a normal cytogenetic segregated into two distinct groups, which also included AML samples from other classes with M1 or M2, was significantly more common in one group, whereas M4 or M5 were more common in the second group. A combination of unsupervised and supervised analysis methods identified a list of genes to predict clinical outcome. The subgroup of samples predicted to have a poor outcome correlated with significantly shorter survival than those samples predicted to have a good outcome. However, it should be noted that when a different analysis algorithm was used ((PAM) (89)) no difference in outcome could be determined, and this may be due a relatively small sample or an inherently poorer performance of patients with a normal cytogenetic.

A second study was published in 2004 (90) on 285 AML patients fully characterized for the presence of mutations within the FLT3 ITD or TKD region, NRAS, KRAS, or CEBPα and the over-expression of EVI1. Data were analyzed by SAM (87) and PAM (89), in addition to using the Pearson correlation algorithms within the OmniViz software package. Their analysis produced 16 distinct groups of patients on the basis of strong similarities in gene-expression profiles. Distinct clusters of t (8;21), inv (16) or t (16;16), and t (15;17) were readily identified, which emphasized the strong effect of these distinctive and recurrent translocations on gene expression. In addition, separate clusters were identified containing samples with monosomy 7, mutations of FLT3, or over-expression of EVI1. In addition, clusters were defined for 11q23 abnormalities or CEPBα mutations. Patients with t (15;17) also clustered into one main group although two subgroups could be identified separating patients with high or low white blood cell count, which also correlated with the presence of FLT3 mutations. Several clusters were not associated with any defined morphological, cytogenetic or mutation status. The best survival rates were for those clusters containing the recurrent translocations (t (8;21), t (15;17) and inv (16) or t (16;16)); these cytogenetic abnormalities have previously been associated with a favorable prognosis (14). Signatures associated with FLT3 ITD were not distinctive; and the authors suggested that this type of mutation, which occurred across the clusters, reflect the heterogeneity of AML.

The molecular characterization of different ALL or AML were analyzed (91) and only a small set of differentially expressed genes was needed to accurately discriminate eight clinically relevant acute leukemia subgroups, including those with t (8;21), t (15;17), t (11q23)/MLL, or inv (16) or t (16;16) , as well as precursor B-ALL with t (9;22), t (8;14), or t (11q23)/MLL and precursor T-ALL. This approach was expanded in a study (92) of 892 patients with mainly AML or ALL, which also included CLL, CML, and some non-leukemia samples. The authors used a combination of unsupervised and supervised statistical methods to identify gene lists that reproduced 12 predefined cytogenetic or morphological leukemia classification groupings, with a 95.1% accuracy, distinct from each other and the non-leukemia samples. Within AML, specificities of 100% were obtained for t (15;17), t (8;21), and inv (16) or t (16;16) ; 97.7% for patients with 11q23 abnormalities and those with complex cytogenetic abnormalities; and 93.7% for the larger and diverse subgroup of "normal cytogenetics and other abnormalities."

4.3. Molecular signatures from mutational abnormalities

As discussed above, several other mutations can occur in AML (FLT3, MLL, CEBPα, or RAS) and are usually observed across FAB, cytogenetic, or WHO subgroups. The heterogeneity of AML masks the identification of a FLT3 specific gene expression profile (88;90). If the heterogeneity is removed by studying only patients with APL (M3 or M3V containing the t (15;17) translocation), then those patients with FLT3 mutations, either ITD or TKD, clearly clustered differently from patients with wild-type FLT3 (63) reinforcing the idea that FLT3 mutations are secondary and may be "over-shadowed" in a heterogeneous AML population.

Some of the larger studies (80;88;90) showed that gene signatures from patients with FLT3, and in particular those for MLL and RAS mutations, were more difficult to identify than for recurrent translocations. Patients with normal cytogenetics, characterized for FLT3-ITD, FLT3-TKD, and NRAS-PM at diagnosis were used to identify a 10-gene signature by neural network analysis that allowed for the correct classification of FLT3-ITD, FLT3-TKD, NRAS-PM, MLL-PTD, and wild-type samples with an accuracy of 83.7% (93). Their data again showed that FLT3-ITD and FLT3-TKD mutations had a distinct clinical phenotype.

Neither did any of the larger studies (88;90) identify any specific cluster associated with MLL PTD nor did a study of pediatric AML, despite clearly identifying the other major cytogenetic types including a subgroup containing patients with rearrangements of the MLL gene (94).

A high proportion of AML with normal cytogenetics have a mutation in the NPM gene and unsupervised clustering which clearly distinguished NPM mutants from NPM wild-type AML, with the expression

profile dominated by a stem cell molecular signature (95). . It is intriguing that a gene signature was identified for the NPMc_ AML group that was not apparently affected by *FLT3* mutation status (95).

4.4 The potential of improving diagnosis with array based signatures

All the studies discussed above are diverse in sample size, patient characteristics, cytogenetic background, and mutational status, but it is reassuring to note that each study has attempted, and in most cases succeeded, to obtain a molecular-based classification. But how relevant are the classifications? For each individual study, they are very relevant, but comparing across studies is perhaps less relevant due to potential differences in extraction procedures, labeling protocols, array platforms, and statistical analyses. Ideally, the data should be harmonized or combined to produce a clear molecular classification of AML based initially on established cytogenetic groupings. The use of microarrays for a global approach to leukemia classification was suggested by Haferlach *et al. (*92) in 2005, in which he stated that a large multi-center comparison assessing microarray diagnosis with standard diagnostics was required. A major step toward this type of diagnostic classification is the launch of an international multi-center clinical research program (MILE (**M**icorarray **I**nnovations in **LE**ukemia) Study) to assess the application of a microarray test and its potential for use in the diagnosis and subclassification of hematologic malignancies. The MILE study research program was launched in 11 centers: 7 European centers in association with the European Leukemia Network (ELN) (96), 3 centers from the United States and one in Singapore. This study will include 4000 patients (from all types of leukemia) in two stages of analysis and has assessed the clinical accuracy of gene expression profiles of 16 acute and chronic leukemia subclasses including MDS with current gold-standard routine diagnostic work-up (97). The advantage of this study is that each participating center will use an identical microarray protocol, laboratory equipment, kits, and reagents for target preparation, hybridization, washing, scanning, and statistical interpretation. The technical failure rate was less than 1%, showing the high level of result reproducibility. Statistical comparison showed a high degree of concordance (~96%) between the gene based result and standard diagnostic procedures. In cases of disagreement, further analysis showed that the the expression array result was usually correct. The second stage of the project will use the Roche "AmpliChip Leukemia" to prospectively validate and determine its diagnostic effectiveness

5. PROGNOSTIC AND THERAPEUTIC SIGNATURES

Current classifications of AML based on cytogenetics and morphology are not robust enough to predict the prognosis for each patient. Broad cytogenetic-based risk groups (14) can be used for stratification, but within these groups are patients with better than expected outcome and, of more concern, those with worse clinical outcome. It was, and still is, the expectation that new prognostic stratification schemes will be formulated based on the global profiling of gene expression identifying genes whose expression is associated with clinical outcome.

The approximately 50% of adult AML patients who have normal cytogenetics (NC) and hierarchical clustering confirmed this diversity as a supervised analysis produced two subgroups: one subgroup which was called a homogeneous or "pure" NC cluster; the other NC-AML had gene signatures that were "translocation like" (83). More significantly, clinical outcome data could discriminate between a poor prognosis group (containing a majority of "pure" NC) and the "translocation-like" AML, which were in the good prognosis class. Another study identified two groups from non-monocytic leukemia, of which one cluster contained all the eight patients that were either refractory to treatment or that relapsed after treatment (84).

A limited number of studies have examined the possible predictive ability of array technologies. One series of childhood AML patients with either FLT3 ITD or TKD mutations identified gene signatures associated with a high risk of treatment failure (98) However, the analysis revealed that the expression levels of two genes (elevated expression of RUNX3 and decreased expression of ATRX) correlated with higher risk of treatment failure. Another study, again of pediatric AML patients, identified a prognostic set of genes, most of which had not previously been linked to prognosis or correlated with FAB or cytogenetics (99).

The use of the profiling software package VxInsight was used to partition 170 AML patients into 6 distinct stable groups based on strong similarities in gene expression and varied significantly in rates of resistant disease; complete response; and disease-free survival (100). Cluster A was dominated by patients with by *NPM1* mutations and had the best overall and disease free survival. In contract, cluster B had the highest proportion of patients with resistant disease. These clusters identified novel groups of AML patients that had not been predicted by traditional studies but have an impact prognosis and potential therapy. This has been reinforced by a study to identify gene expression markers that predict response to the orally available farnesyltransferase inhibitor tipifarnib (Zarnestra) in AML (101). Supervised statistical analysis identified only eight gene expression markers that could predict patient response however, the overall accuracies were only around 60-65%, but showed that in principle, gene expression profiles may be able to identify specific groups of patients that might respond to targeted therapies.

6. miRNA SIGNATURES

microRNAs (miRNAs) are small non-coding RNAs of 20–22 nucleotides, typically excised from 60–110 nucleotide foldback RNA precursor structures (102) miRNAs are involved in crucial biological processes, including development, differentiation, apoptosis and proliferation (102) through imperfect pairing with target messenger RNAs (mRNAs) of protein-coding genes and

the transcriptional or post-transcriptional regulation of their expression (103).

Many tumour types have been analysed by miRNA profiling and each has shown differing miRNA profiles when compared with normal cells from the same tissue (104;105). In one of these profiling studies genome-wide miRNA expression was done in a systematic analysis of 334 leukaemias and solid cancers (105). In addition to showing that miRNA levels were higher in normal tissues compared to tumors, they also reported that miRNA expression profiles could classify human cancers according to the developmental lineage and differentiation state of the tumours. The small number of AML samples that were analysed clustered together within a larger cluster of comprising B- and T-acute lymphoblastic leukaemia and lymphoma samples. In patients with t (15;17) translocations, the post-transcriptional regulation by miR-223 may be important for granulocytic differentiation and clinical response of acute promyelocytic leukemia (APL) blasts to all-trans retinoic acid (ATRA) (106), whilst the effect of ATRA on cell differentiation was shown to correlate with the activation of known miRNA regulators (107). In a study on human leukaemia cell lines, specific miRNA clusters or individual species were identified that were involved in the maintainance of lineage specification (108).

Recently, several miRNA species were examined using a quanitative RT-PCR approach from a group of primary AML patients with a normal karyotype (109). The expression levels of each miRNA were correlated with the mRNA expression micro-arry profiles obtained from the same leukaemia sub-group. Using Pearson or Spearman statistical correlations, list of genes with very high levels of significance were obtained and showed that miR-181a in particular, has a strong correlation with AML with a normal karyotype sub-group. Whilst other specific miRNAs were shown to have a clear and significant correlation with HOX gene expression.

Studies have shown that the determination of miRNA expression, either by quantitative PCR or array, may also have diagnostic, prognostic or therapeutic potential, either alone or in combination with mRNA expression signatures.

6. DISCUSSION

The genomics of AML is one of the fastest moving areas of cancer research. It is probably one of the best-understood malignant diseases, and its dissection and understanding of the molecular abnormalities can be used as a paradigm for numerous other diseases. Mutations that block differentiation combine with those that promote proliferation or survival to form the heterogeneous spectrum of phenotypes seen in AML (110). These mechanisms are now being dissected from all angles including miRNA and mRNA expression profiling which will lead to developments for diagnosis, prognosis, clinical trials and therapeutic interventions.

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Abbreviations: AML : Acute myeloid leukaemia; FAB: French-American-British; WHO: World Health Organisation; ITD: internal tandem duplication; TKD: tyosine kinase domain

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