

Stratification of Acute Myeloid Leukemia Based on Gene Expression Profiles

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Abstract

Acute myeloid leukemia (AML) is characterized by clonal growth of immature leukemic blasts and develops either de novo or secondarily to anticancer treatment or to other hematologic disorders. Given that the current classification of AML, which is based on blast karyotype and morphology, is not sufficiently robust to predict the prognosis of each affected individual, new stratification schemes that are of better prognostic value are needed. Global profiling of gene expression in AML blasts has the potential both to identify a small number of genes whose expression is associated with clinical outcome and to provide insight into the molecular pathogenesis of this condition. Emerging genomics tools, especially DNA microarray analysis, have been applied in attempts to isolate new molecular markers for the differential diagnosis of AML and to identify genes that contribute to leukemogenesis. Progress in bioinformatics has also yielded means with which to classify patients according to clinical parameters such as long-term prognosis. The application of such analysis to large sets of gene expression data has begun to provide the basis for a new AML classification that is more powerful with regard to prediction of prognosis.

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Key words: DNA microarray; CD133; CD34; Transcriptome

1. Introduction

The human genome project was launched in 1991 as a joint program by the United States, the United Kingdom, Japan, France, Germany, and China. Ten years later, the first draft sequence of the entire human genome, including the nucleotide sequence of approximately 90% of euchromatin with 99.9% accuracy, was completed by the international team [1]. The total number of protein-coding genes was estimated to be approximately 31,000, only twice that for a nematode [2]. Completion of the human sequencing project was announced in 2003, with the determined nucleotide sequence encompassing >99% of euchromatin with an accuracy of 99.99% (<http://www.ncbi.nlm.nih.gov/genome/guide/human>). Annotation of the final sequence is still in progress but is expected to be completed soon. The postgenome era is therefore about to begin.

Compilation of the catalog of human genes has spurred the development of new technologies to investigate and characterize changes—at the gene, messenger RNA (mRNA), or protein level—in the entire gene set simultaneously. Among

such genomics approaches, DNA microarray analysis has probably been the most successful to date. A DNA microarray resembles a microscope slide and contains tens of thousands of genomic fragments, complementary DNAs (cDNAs), or oligonucleotides present in individual spots. Hybridization of such arrays with labeled cDNAs derived from a sample mRNA population allows measurement of the amounts of the original mRNAs for all genes represented on the array [3]. The high density of DNA fragments achievable on currently available microarrays makes it possible to quantitate the mRNA levels for all human genes with 1 array experiment. Such profiling of gene expression in a given cell or tissue type promises to provide a new dimension to our understanding of biology.

Microarray analysis has been applied, for instance, to characterize the differentiation [4] and the inflammatory response [5] of human cells. Gene expression profiling has also helped to develop new classification systems for human diseases that had previously been categorized on the basis of pathology or cell morphology. Microarray analysis of human specimens of prostate cancer, for example, has resulted in the identification of new biomarkers that predict poor prognosis [6]. Characteristic patterns of gene expression, or “molecular signatures,” are similarly expected to provide a basis for the subdivision of patients with the same clinical diagnosis into groups with distinct prognoses [7].

Acute myeloid leukemia (AML) is characterized by the clonal growth of immature leukemic blasts in bone marrow

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(BM) and can develop de novo or from either myelodysplastic syndrome (MDS) or anticancer treatment [8]. One of the most robust predictors of AML prognosis is blast karyotype [9,10]. A good prognosis is thus predicted from the presence in leukemic clones of t(8;21), t(15;17), or inv(16) chromosomal rearrangements, whereas -7/7q-, 11q23, or more complex abnormalities are indicative of a poor outcome. Such stratification is not informative, however, for predicting the prognoses of patients with a normal karyotype, who constitute approximately 50% of the AML population.

A clinical record of a preceding MDS phase is also an indicator of poor prognosis for individuals with AML. Therapy-related acute leukemia (TRL) can develop after the administration of alkylating agents, topoisomerase inhibitors, or radiotherapy. The clinical outcome of TRL is generally worse than that of de novo AML [11], and a subset of individuals with TRL also exhibits multilineage dysplasia of blood cells. Prediction of the outcome of and optimization of the treatment for each AML patient would thus be facilitated by the ability to differentiate de novo AML from MDS-related AML and TRL. However, dysplastic changes (in particular, dyserythropoiesis) in differentiated blood cells are also found not infrequently in the BM of healthy elderly individuals [12]. The differential diagnosis among AML-related disorders is therefore not always an easy task in the clinical setting, especially if a prior record of hematopoietic parameters is not available.

The application of DNA microarray analysis to AML has the potential (1) to identify molecular markers for the differential diagnosis of AML-related disorders, (2) to provide a basis for the subclassification of such disorders, and (3) to yield insight into the molecular pathogenesis of AML. In this article, I review progress related to the first 2 of these goals.

2. Identification of Molecular Markers for the Differential Diagnosis of AML

2.1. Karyotype

Given that the current classification of AML relies on blast karyotype, it would be informative to determine whether karyotype is related to the gene expression profile of blasts. In other words, is DNA microarray analysis able to substitute for conventional karyotyping?

Schoch et al performed microarray analysis with BM mononuclear cells (MNCs) isolated from individuals with AML and compared the data among the patients with t(8;21), t(15;17), or inv(16) chromosomal anomalies [13]. Each of these 3 AML subgroups was found to possess a distinct molecular signature, and it was possible to predict the karyotype correctly on the basis of the expression level of specific genes. The leukemic blasts of these subgroups of AML manifest distinct differentiation abilities, however. Blasts with t(8;21) remain as immature myeloblasts, those with t(15;17) differentiate into promyelocytes, and those with inv(16) differentiate into cells of the monocytic lineage. The overall gene expression profiles of these 3 types of blasts therefore might be substantially affected by the mRNA repertoires of the differentiated cells present within BM. It remains to be determined whether such "karyotype-specific"

molecular signatures are indeed dependent on karyotype or are related to French-American-British (FAB) subtype (differentiation ability).

The gene expression profiles of BM MNCs derived from a large number of pediatric AML patients were examined by Yagi et al [14]. Clustering of these patients according to the expression pattern of the entire gene set resulted in their separation into FAB subtype-matched groups, indicative of a prominent influence of differentiated cells within BM on the gene expression profile. It may nevertheless prove possible to capture bona fide karyotype-dependent genes from large data sets with the use of sophisticated bioinformatics approaches and then to use the expression profiles of these genes for "pseudokaryotyping."

Virtaneva et al purified CD34⁺ progenitor cells from the BM of individuals with AML and compared the gene expression profiles of the patients with a normal karyotype and those with trisomy 8 [15]. They also compared such AML blasts with CD34⁺ fractions isolated from the BM of healthy volunteers. The use of CD34⁺ (immature) cells for microarray analysis would be expected to reduce the influence of differentiated cells within BM on the overall pattern of gene expression. These researchers found that the AML blasts differed markedly from normal CD34⁺ cells in terms of the gene expression profile. However, the blasts with trisomy 8 did not appear to differ substantially from those with a normal karyotype. It is possible that blasts with a normal karyotype or those with trisomy 8 are too diverse to allow identification of distinguishing gene markers.

2.2. De Novo AML versus MDS-Related AML

Although dysplasia is the diagnostic hallmark of MDS, such abnormal cell morphology is also associated with other conditions, and the subjective assessment of the extent of dysplasia suffers from the risk of variability from physician to physician. It is therefore desirable to identify molecular markers that are able to distinguish de novo AML from MDS-associated AML. It is also important to clarify whether de novo AML and MDS-associated AML are indeed distinct clinical entities or whether they overlap to some degree.

Although DNA microarray analysis is a promising tool for the identification of such molecular markers able to differentiate de novo AML from MDS-related AML, a simple comparison of BM MNCs for these 2 conditions is likely to be problematic. The cellular composition of BM MNCs differs markedly among individuals. Differences in the gene expression profile between BM MNCs from a given pair of individuals may thus reflect these differences in cell composition [16]. The elimination of such pseudopositive and pseudonegative data necessitates the purification of background-matched cell fractions from the clinical specimens before microarray analysis.

Given that de novo AML and MDS both result from the transformation of hematopoietic stem cell (HSC) clones, HSCs would be expected to be an appropriate target for purification and gene expression analysis. With the use of an affinity purification procedure based on the HSC-specific surface protein CD133, also known as AC133 [17], we have purified CD133⁺ HSC-like fractions from individuals with

various hematopoietic disorders, and we have stored these fractions in a cell depository referred to as the *Blast Bank*. With such background-matched purified samples, we attempted to identify differences in gene expression profiles between de novo AML and MDS-associated AML [18]. To minimize further the influence of differentiation commitment of blasts toward certain lineages, we used only Blast Bank samples with the same phenotype, the M2 subtype according to the FAB classification. We thus characterized the expression profiles of >12,000 genes in CD133⁺ Blast Bank samples from 10 patients with de novo AML of the M2 subtype as well as from 10 individuals with MDS-related AML of the same FAB subtype.

Selection with a Student *t* test ($P < .01$) and an effect size of ≥ 5 units for discrimination between the 2 clinical conditions led to the identification of 57 “diagnosis-associated genes,” the expression profiles of which are shown in a “gene tree” format in Figure 1A. In this format, genes with similar expression patterns across the samples are clustered near each other. Patients were also clustered in this tree (2-way clustering) on the basis of the similarity of the expression pattern of the 57 genes (dendrogram at the top). All subjects were clustered into 2 major groups, 1 composed mostly of de novo AML patients and the other containing predominantly patients with MDS-associated AML. Each of these 2 main branches contained misclassified samples, however, indicating that simple clustering was not sufficiently powerful to differentiate the 2 clinical conditions completely. Furthermore, this analysis might not adequately address whether de novo AML and MDS-associated AML should be treated as distinct entities, at least from the point of view of the gene expression profile.

Decomposition of the multidimensionality of gene expression profiles by the application of principal components analysis [19] or correspondence analysis [20], for example, is often informative for such purposes. Application of the latter method to the data set of the 57 genes reduced the number of dimensions from 57 to 3. On the basis of the calculated 3-dimensional (3D) coordinates for each sample, the specimens were then projected into a virtual 3D space (Figure 1B). Most of the de novo AML samples were positioned in a region of this space that was distinct from that occupied by the MDS-associated AML specimens. However, 2 of the former samples were localized within the MDS region. These results suggest that de novo AML and MDS-associated AML are distinct disorders but that the current clinical diagnostic system is not efficient enough to separate them completely.

Instead of extracting a molecular signature from the expression profile of multiple genes, an alternative approach is to attempt to identify individual gene markers specific to either de novo AML or MDS-associated AML. We used the Blast Bank array data to identify MDS-specific markers, defined as genes that are silent in blasts from all de novo AML patients but are active in those from at least some patients with MDS-related AML [16]. This approach resulted in the identification of a single gene, *DLK*, that matched these criteria. *DLK* is expressed in immature cells [21] and is implicated in the maintenance of the undifferentiated state [22]. Selective expression of *DLK* in MDS blasts may thus contribute to the pathogenesis of MDS. Increased expression

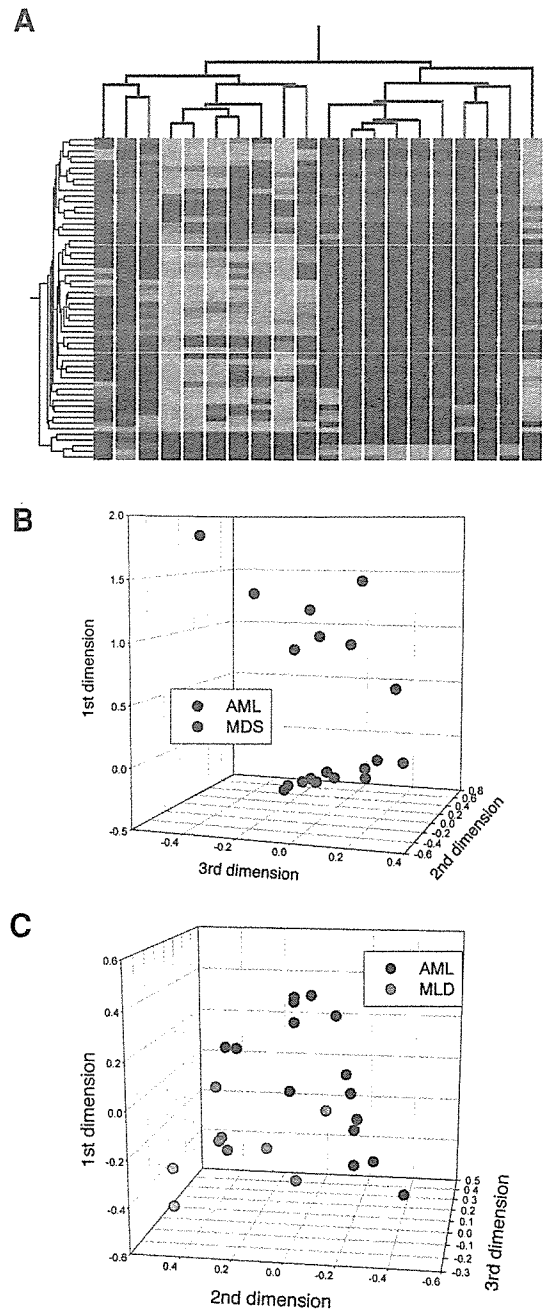


Figure 1. Correspondence analysis and 3-dimensional (3D) projection for differential diagnosis of acute myeloid leukemia (AML). A, Two-way hierarchical clustering for 57 disease-associated genes and 20 patients. Each row corresponds to a single gene and each column to CD133⁺ cells from a patient with de novo AML (blue) or myelodysplastic syndrome (MDS)-related AML (red). The expression level of each gene is color coded, with a high level in red and a low level in green. B, Projection of the 20 specimens from (A) into a virtual space with 3 dimensions identified by correspondence analysis of the 57 genes. Patients with de novo AML (AML) were separated from those with MDS-related leukemia (MDS). C, Projection of specimens from patients with de novo AML without dysplasia (AML) or de novo AML with multilineage dysplasia (MLD) into a 3-dimensional space based on correspondence analysis of differences in gene expression. The dendrogram in (A) and 3-dimensional projections in (B) and (C) were constructed from data in [18] and [28].

of *DLK* in blasts from individuals with MDS has also been demonstrated in other studies [18,23].

Cell fractions other than CD133⁺ cells are also potential targets for microarray analysis. Pellagatti et al chose peripheral blood neutrophils to investigate the gene expression profiles of MDS and MDS-associated AML [24]. They identified genes whose expression was dependent on MDS subtype. Given the high activity of ribonuclease in neutrophils, however, it is important to confirm the reproducibility of expression data obtained with these cells.

2.3. Dysplasia

In addition to the dysplastic blood cells associated with MDS, prominent dysplasia is apparent in certain individuals with de novo AML for whom the possibility of a prior MDS phase can be excluded [25,26]. Such de novo AML with dysplasia has a poor outcome with conventional chemotherapy [27], as does MDS-related leukemia. In the revised classification of AML by the World Health Organization [8], an entity of AML with multilineage dysplasia (AML-MLD) has been proposed; this entity probably includes both de novo AML with dysplasia and AML secondary to MDS. Whether such an amalgamation has clinical relevance awaits further studies.

To clarify directly whether de novo AML-MLD is indeed a clinical entity distinct from de novo AML without dysplasia, we searched for differences between the transcriptomes of CD133⁺ cells derived from individuals with diagnoses of these 2 conditions [28]. We attempted to construct a 3D view of the samples with the coordinates calculated from correspondence analysis of genes found to be associated with dysplasia (Figure 1C). Most cases of AML-MLD were separated from those of AML without dysplasia in the 3D space. In contrast to the prominent separation power of the first dimension in Figure 1B, both the first and second dimensions substantially contributed to separation of the samples in Figure 1C. These data indicate that de novo AML without dysplasia can be differentiated from de novo AML-MLD on the basis of gene expression profiles.

3. Stratification of AML

3.1. Analysis of BM MNCs

Given that the current classification of AML is not sufficiently powerful to predict the prognosis of each affected individual, it is hoped that DNA microarray analysis will provide a better stratification scheme to separate AML patients into prognosis-dependent subgroups. To this end, Bullinger et al isolated MNCs from either BM or peripheral blood of 116 adult AML patients and examined the gene expression profiles of these cells with DNA microarrays harboring >39,000 cDNAs [29]. From the data set, they then screened "class predictor" genes, whose expression was correlated with the duration of patient survival. Class prediction based on such genes separated the patients into 2 classes, and long-term survival differed significantly between these 2 classes for both the training set ($P < .001$, log-rank test) and the test set ($P = .006$). This expression profile-based classification

also separated AML patients with normal karyotype into 2 classes with distinct prognoses ($P = .046$). Although the number of genes used for the class prediction was relatively large ($n = 133$), these data demonstrated that DNA microarray analysis is able to predict the prognosis of AML patients in a manner independent of karyotype.

Similarly, Valk et al measured the expression levels of approximately 13,000 genes in BM MNCs isolated from 285 patients with AML [30]. Unsupervised clustering based on the gene expression profiles separated the patients into 16 subgroups. The prognoses of patients differed among these clusters, but whether the ability to predict prognosis was independent of karyotype was not addressed.

The gene expression profiles of BM MNCs from 54 pediatric AML patients were compared for those individuals who maintained complete remission (CR) for >3 years and those who failed to enter initial CR [14]. Thirty-five genes were identified as associated with prognosis and were used to separate the individuals into 2 groups. The difference in survival between the 2 groups was again statistically significant ($P = .03$), although whether this approach was independent of the current classification system was not addressed.

3.2. Analysis of Purified Fractions

As described in section 2.2, microarray analysis of purified fractions is likely to be more accurate than is that of BM MNCs for the extraction of prognosis-associated molecular signatures. We have analyzed the expression intensities of approximately 33,000 genes (likely representing almost the entire human genome) in CD133⁺ HSC-like fractions isolated from 66 patients with AML who received standard chemotherapy. Of these patients, 51 individuals entered initial CR, whereas the remaining 15 failed to do so. Comparison of the data set for these 2 classes resulted in the identification of a small number of outcome-related genes (Yamashita et al, unpublished data). Principal components were extracted from the gene expression patterns, and the patients were projected into a virtual 3D space based on the coordinates obtained by correspondence analysis. Individuals who entered CR were separated from those who did not (Figure 2A), indicating that the gene expression profile of leukemic blasts indeed differs between AML patients who respond to chemotherapy and those who do not. In this analysis, the separation of the 2 groups of patients was achieved mostly in the first dimension. We therefore separated the patients into 2 subgroups according to whether the coordinate in the first dimension was < -0.3 or ≥ -0.3 . The individuals in the latter group lived significantly longer than did those in the former (Figure 2B). These data support the feasibility of a novel stratification scheme for AML based on the gene expression profile.

4. Conclusion

Newly developed genomics tools allow global assessment of mRNA levels, DNA copy number, or genomic polymorphisms. Among these tools, DNA microarray analysis has proved highly successful in studies of human specimens, not only from individuals with hematopoietic disorders but also

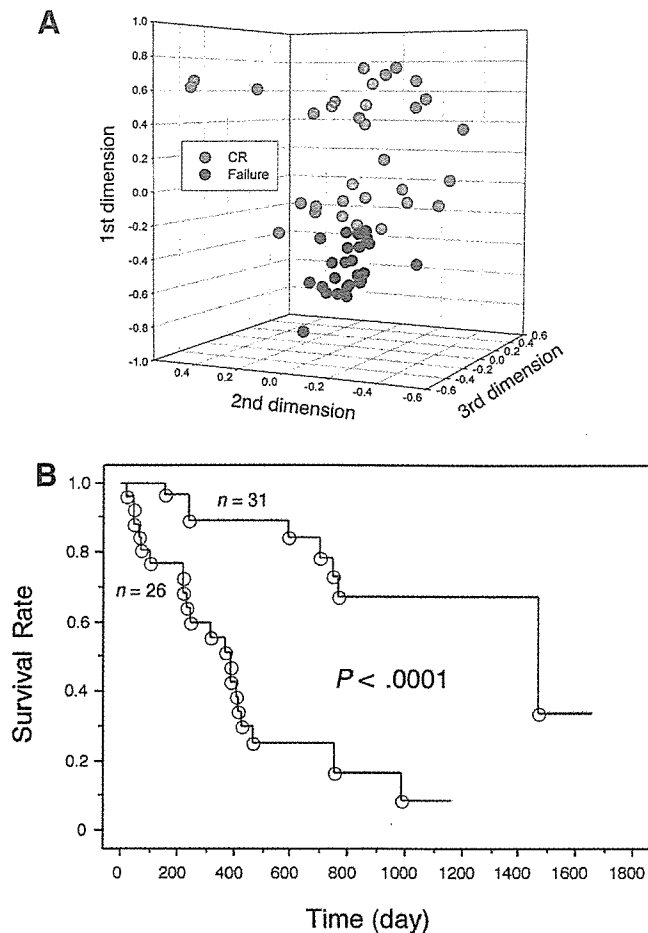


Figure 2. Stratification of acute myeloid leukemia (AML) on the basis of microarray analysis of gene expression. A, Projection of specimens from patients with AML who underwent initial chemotherapy-induced complete remission (CR) and those who did not (Failure) into a 3-dimensional space based on correspondence analysis of differences in gene expression. B, Kaplan-Meier analysis of the subjects in (A), revealing significantly different prognoses for the 2 classes with a coordinate in the first dimension of <-0.3 or ≥-0.3 .

from those with solid tumors or nonmalignant degenerative diseases. Insight into many more human disorders is likely soon to be provided by such global profiling of gene expression. It is important to bear in mind, however, that DNA microarray data are prone to contamination with pseudopositive or pseudonegative results. The efficient characterization of AML thus appears to require optimal purification of target cell populations. Provided that experiments are designed carefully, DNA microarray analysis is likely to shed new light on the molecular pathogenesis of AML.

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5. 多発性骨髄腫と関連疾患の遺伝子発現プロファイル

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key words DNA microarray, multiple myeloma, MGUS, CD138

動 向

多発性骨髄腫 (multiple myeloma: MM) は形質細胞の異常増殖を本体とする悪性疾患であり高齢者に好発する¹⁾。MMはきわめて予後不良であり、ほとんどの例において最終的には化学療法に抵抗性となる²⁾。一方、以前は良性単クローン性免疫グロブリン血症とよばれていた monoclonal gammopathy of undetermined significance (MGUS) は、MMに比べて骨髄中の形質細胞の割合が少なく、また一般に全身症状も伴わない。しかしMGUS患者の中には1年に1%程度の割合でMMへ移行する症例が存在しており、MGUSとMMとの異同はまだまだ不明確である³⁾。MGUSは前癌状態ともいうべきものなのか、それともMGUSは不均一な集団であり一部の特徴的なサブグループのみがMMへ移行するのか、またMGUSとMMとを区別する genetic events は果たして何なのか? など疑問点が多い。

一方、MMも発症当初は化学療法が比較的奏効し、一旦は症状が軽減するが、化学療法自体でMM細胞が根治されることはほとんどなく、早晩、患者骨髄腫細胞は化学療法に抵抗性となる。現段階でMMの生命予後を明確に改善する治療法は骨髄移植のみであり⁴⁾、それも自家と同種のどちらが優れているかは議論の多いところであ

る⁵⁾。

比較的罹患人口の多い疾患にもかかわらず、MMの病態はほとんど不明なままである。このようなMMの発症機構を明らかにし、同時に各患者の予後を予測することを目的として、DNAチップを用いた新しいアプローチが複数の施設から報告されるようになった。これらの解析結果はいまだ必ずしも画期的とはいえないが、MMに関して全く新しい側面からの知見が急速に蓄積されつつあることは間違いない。本稿ではMM細胞の起源あるいはMM患者のサブグループの提言など、これらのチップ解析の結果得られた情報について概説したい。

A. 遺伝子発現プロファイル

ヒトゲノムプロジェクトの成果であるドラフトシーケンスが2001年2月に公表され^{6,7)}、またついに euchromatin の完全版配列決定が2003年4月14日に宣言された。現在、得られた配列上に遺伝子の割付作業が行われているが、近い将来にその成果が公表されると思われる。ヒトのほとんどの遺伝子構造が(機能は不明であるにしろ)明らかになった今後の「ポスト・ゲノム時代」においては医療・医学研究の分野でも方法論の転換が

求められるであろう。たとえばある疾患の原因遺伝子を同定することを考えるとき、旧来であれば発現スクリーニング法や連鎖解析法などによって全く未知の遺伝子を最初から同定せざるを得なかった。しかしポスト・ゲノム時代においては理論上「未知」の遺伝子は存在しなくなり、膨大ではあるがあくまで有限なヒト遺伝子のプールの中から特定の特徴を備えた遺伝子を効率よく同定することが必要になる。このような網羅的解析は、DNA シークエンス、遺伝子発現量 (mRNA 量) および蛋白質の全ての段階で行われるであろう。この中で現在すでに強力な網羅的解析技術が存在するのは「遺伝子発現量」の分野であり、その技術とは DNA チップである。

DNA チップはスライドガラスなどの担体の上に、cDNA あるいは遺伝子由来のオリゴヌクレオチドを高密度に配置したものであり、スライド上の数千から数万種類の遺伝子の相対的発現量を一度の実験で解析することができる⁸⁾。すでにデータベース上に存在するヒトの全予想遺伝子を配置した DNA チップも市販されており (<http://www.affymetrix.com>)、これら的高密度 DNA チップを用いることで、たとえば任意の細胞・組織における全遺伝子の発現パターン (遺伝子発現プロファイル) も比較的簡便に把握可能となる。gene に「全体」を表す「-ome」という接尾語を付けて genome (ゲノム) という単語が作られたように、transcript に -ome を付加した transcriptome (トランスクリプトーム) という造語も最近是用いられるようになった。

このような遺伝子発現プロファイル・トランスクリプトームをたとえば MM と MGUS 間で大規模に比較することで、両者を鑑別可能な新しい分子診断マーカーの同定も可能になると期待される。また DNA チップによって得られる発現データを用いて、疾患の分類自体に新しい体系を導入することも可能であろう。

B. CD138 陽性分画を用いた比較

しかし、たとえば健常者と MM 患者骨髄単核球 (mononuclear cell: MNC) を単純に比較するような DNA チップ実験を行うと、意外にも本アッセイが偽陽性の多い解析法であることに気づく。形質細胞は健常者骨髄中で数%を占めるに過ぎないが、MM 患者においては時に数十%に及ぶ。したがって骨髄 MNC 間で比較すると、形質細胞固有な遺伝子は MM 患者において見かけ上必ず発現が誘導されているように結論づけられてしまうのである。このような偽陽性結果を回避するためには、疾患責任分画である形質細胞のみを様々な患者より純化した上で、DNA チップによって比較することが重要であろう。B リンパ球は成熟形質細胞への分化に伴い細胞表面 CD19、CD20 の発現量が低下し、逆に CD138、CD38 の発現が誘導される。特に CD138 は骨髄中の血球において成熟形質細胞にのみ発現しており、同細胞分画を純化するのに好適である⁹⁾。

そこで我々を含め複数の研究グループにおいて、健常者、MGUS 患者及び MM 患者骨髄より CD138 陽性形質細胞を純化し DNA チップによって比較するプロジェクトが進行した。具体的な CD138 陽性細胞の純化ステップとして、抗 CD138 抗体に磁気ビーズを結合させた純化カラムが用いられている。フローサイトメーターを用いた細胞分画の純化も可能であるが、たとえば 1×10^6 個の CD138 陽性細胞を FACS によって純化するのは長時間を要する。それに比べて磁気ビーズカラムを用いれば同程度の細胞の純化も 1 時間以内に回収可能である。磁気ビーズカラムによる細胞純化の効率を図 1 に示す。たとえば本解析における健常者の骨髄 MNC 中 CD138 陽性細胞は 1.8% しか存在しないが、同じ健常者 MNC を純化カラムにかけると最終的に CD138 陽性細胞 94.9% のきわめて純度の高い形質細胞分画が得られるの

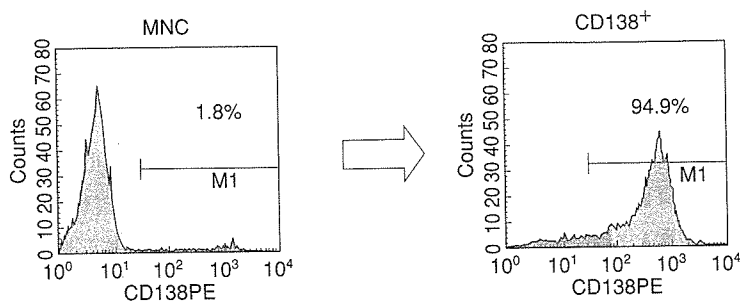


図1 CD138 陽性細胞の純化

健常者より単核球 (MNC) を純化し、さらに抗CD138抗体を用いた磁気ビーズカラムによりCD138陽性細胞 (CD138⁺) を純化した。両分画に対してFITC結合抗CD138抗体によるFACS解析を行ったヒストグラムを表す。それぞれにおけるCD138陽性細胞の割合も%で示してある。

である。同分画のサイトスピン標本をWright-Giemsa染色にて鏡検すると、均質な形質細胞のみが純化されていることが確認された。

C. 多発性骨髄腫の解析

1. 免疫グロブリンサブタイプでの比較

Magrangeasらは、88例のMM患者および9例の形質細胞性白血病 (plasma cell leukemia: PCL) 患者骨髄よりCD138陽性形質細胞を純化し、各サンプルより放射標識したcDNAを作成してカスタムメイドのcDNAマクロアレイ (5376遺伝子) にハイブリダイズさせた¹⁰⁾。得られた遺伝子発現プロファイルをMMの異常免疫グロブリンのサブタイプ間で比較している。たとえば産生グロブリンがIgGであるもの (48例) とIgA (21例) とで比較し、両者を識別する発現プロファイルをもつ遺伝子群を抽出した。これらには免疫グロブリン関連遺伝子だけでなく、GATA6やLKF1等の転写因子、またBRCA1等のがん抑制遺伝子も含まれていた。またこれらサブタイプ依存性遺伝子セットにおける遺伝子発現パターンの類似性から、サンプルの系統樹を作成するとBence Jones proteinタイプのMM患者はIgG、

IgA産生型いずれとも異なる別の疾患グループを形成することがわかった。

また彼らは産生される免疫グロブリンのL鎖のサブタイプによる比較も行っている。λ鎖産生MMとκ鎖産生型との間で発現量に偏りがある遺伝子を抽出したところ、κ鎖産生MM細胞においてMIP1α¹¹⁾、TGFβ₃、BMP2などの骨代謝に関係する遺伝子群が特異的に発現していることが示された。一方、λ鎖型ではTGFβ機能を抑制するLTBP4遺伝子が特異的に発現することが示された (表1)。これらはMM細胞で産生されるL鎖のタイプ依存性に骨病変の重症度が異なる可能性を示唆する。そこでこれらL鎖依存性遺伝子の発現プロファイルを元に患者サンプルを並び替え、系統樹を作成すると、図2に示されるように骨病変が軽症の患者サンプルの多くはλ鎖産生グループに分類され、一方、骨病変が重症な患者サンプルの多くはκ鎖グループに分類されることが明らかになった。これまでL鎖のサブタイプがMM患者における骨融解の程度にリンクするとの臨床情報があったが¹²⁾、Magrangeasらのデータは、分子生物学的な裏づけを与えるものとして興味深い。

表1 λ 鎖型と κ 鎖型で発現が異なる遺伝子¹⁰⁾

gene symbol	gene description	accession no.	discriminating score
<i>IGL@</i>	Ig λ	H14524	- 2.74
<i>IGL@</i>	Ig λ	R83196	- 1.81
<i>CDSN</i>	corneodesmosin	W95594	- 1.77
<i>MCP</i>	membrane cofactor protein (CD46)	H26673	- 1.66
<i>IGL@</i>	Ig λ	H15030	- 1.62
<i>ABCD4</i>	ATP-binding cassette, subfamily D, member 4	H51632	- 1.22
<i>SAMHD1</i>	SAM domain and HD domain, 1	H47862	- 1.07
<i>LTBP4</i>	latent transforming growth factor- β -binding protein 4	R73631	- 1.02
<i>TGF3</i>	transforming growth factor, β -3	W80655	0.73
<i>TNFRSF6</i>	tumor necrosis factor receptor superfamily, member 6 (CD95)	AA031300	1.06
<i>BMP2</i>	bone morphogenetic protein 2	AA114112	1.34
<i>PRKCABP</i>	PRKCA-binding protein	AA641722	1.54
<i>HOX11</i>	homeobox 11	AA007444	1.61
<i>EXT1</i>	exostoses, multiple, type 1	R13402	1.70
<i>IGKC</i>	Ig κ	R71916	1.82

discriminating scoreは両群における平均値の差を標準偏差の差で割ったもので、両群間における発現量の偏りを表す。

2. 疾患サブタイプでの比較

Zhanらは、健常者31例、MGUS患者5例およびMM患者74例の骨髄よりCD138陽性形質細胞分画を純化し、Affymetrix社のGeneChip HuGeneFLチップ(～6800遺伝子)による解析を行った。また同様に8種類の多発性骨髄腫細胞株についてもチップ解析を行っている。これら計118例のチップデータを基にサンプル全体の系統樹を作成すると、健常者は「MGUS + MM + 細胞株」群とは異なるグループを形成することが示され、遺伝子発現プロファイルによる疾患解析の妥当性が支持される結果となった¹³⁾。

さらにMM内でのみサンプルの系統樹を作成すると、図3に示されるように大きく4種類の患者サブグループ(MM1～MM4)に分かれることが明らかになった。しかもMM1はMGUSに最も近い遺伝子発現プロファイルをもち、MM4は骨髄腫細胞株に最も近似したプロファイルをもつことも示された。すなわちMM内のこれらサブタイプが患者の予後にリンクする可能性が示唆される。実際MM1とMM4タイプ間で最も発現量

が異なる遺伝子をリストすると、DNA複製や修復に関わる遺伝子群[thymidylate synthase (TYMS), mutS homolog 2 (MSH2)等]がMM4グループに特異的に発現していることがわかる(表2)。これらのデータはMM4に属する骨髄腫細胞の増殖能力が亢進していることを支持するといえよう。またMM患者における既知の予後不良因子である血中 β_2 ミクログロブリン¹⁴⁾、クレアチニンレベルの増加は、ともにMM4群において顕著なことも確認された。

Zhanらは、同じデータセットから正常形質細胞とMM細胞との比較も行っている。たとえば骨髄腫の細胞において特異的に発現する遺伝子として、cyclin-dependent kinase inhibitor 1A, c-Ablキナーゼ等が疾患特異的に発現亢進していることが確認された。以上の実験結果から、遺伝子発現プロファイルによって正常形質細胞と異常(MGUS + MM)を区別することは可能であったが、MGUSとMMとを正確に判別することは困難であると結論づけられた。

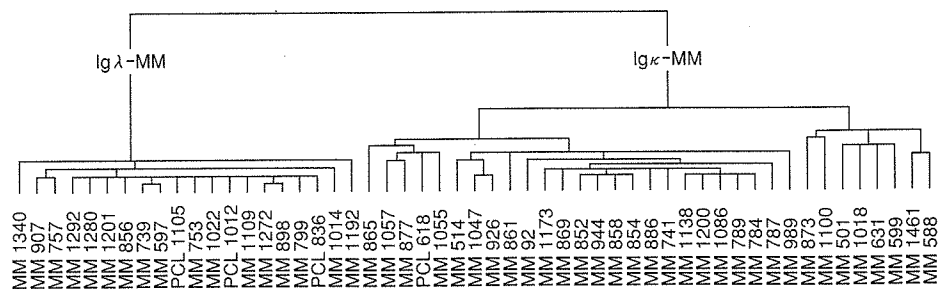


図2 L鎖サブタイプと骨病変 (文献10より改変)

産生免疫グロブリンのL鎖サブタイプ依存性遺伝子群の発現プロファイルを基に患者の系統樹を作成した。解析した患者の中で骨病変がないか軽微なサンプルを青色で、また骨病変が重度の患者を黒色で表す。

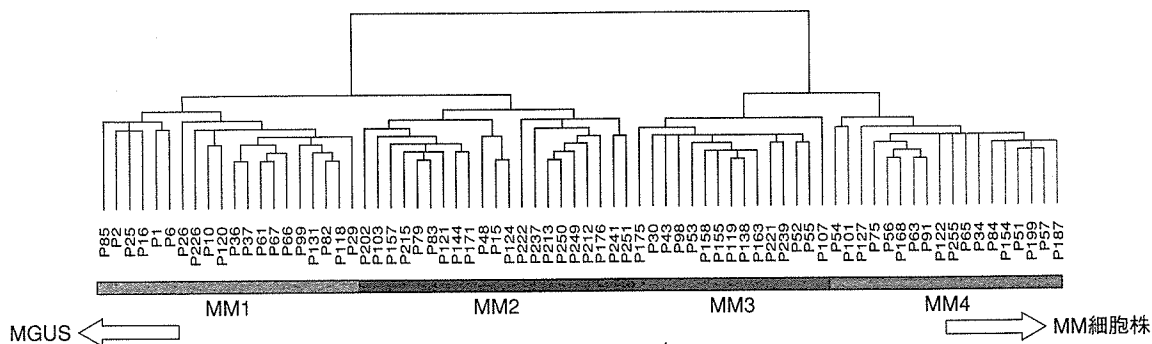


図3 MM患者サンプルの遺伝子発現プロファイルによる系統樹 (文献13より改変)

MM患者74例の骨髄CD138陽性細胞における遺伝子発現プロファイルを基にサンプルの系統樹を作製したところ、MM1～MM4までの4種類のサブタイプに大きく分類されることが示された。なおMM1はMGUSのCD138陽性細胞に似た発現プロファイルをもち、MM4は骨髄腫細胞株群と似たプロファイルをもつ。

表2 M1～M4間で発現量が異なる遺伝子¹³⁾

accession no.	function	gene symbol	MM1 (N=20) P	MM4 (N=18) P	χ^2	WRS P value
D00596	DNA replication	TYMS	3	18	24.35	1.26×10^4
U35835	DNA repair	PRKDC	2	17	23.75	4.55×10^6
U77949	DNA replication	CDC6	1	13	15.62	5.14×10^6
U91985	DNA fragmentation	DFFA	1	12	13.38	6.26×10^5
U61145	transcription	EZH2	4	15	12.77	1.67×10^4
U20979	DNA replication	CHAF1A	2	12	10.75	1.10×10^4
U03911	DNA repair	MSH2	0	9	10.48	2.88×10^6
X74330	DNA replication	PRIM1	0	9	10.48	9.36×10^6
X12517	SnRNP	SNRPC	0	9	10.48	5.26×10^6
D85131	transcription	MAZ	0	9	10.48	1.08×10^5
L00634	farnesyltransferase	FNTA	10	18	9.77	7.28×10^5

WRS: Wilcoxin rank sum test

D. 形質細胞の分化様式

Bリンパ球は最終的に形質細胞へと分化成熟し免疫グロブリン蛋白を大量に産生ようになる。しかし初期のBリンパ球発生が比較的詳細に検討されてきたのに比し、最終段階である形質細胞への分化がどのようなメカニズムによって調整されているかはほとんど知られていない。このようなBリンパ球の発達様式の解明にもDNAチップは強力なツールとなり得るであろうし、またそのような知見はMM細胞の起源にも重要な情報を与えるであろう。

扁桃でのBリンパ球は同じ組織内でCD138陽性形質細胞へと分化し、やがて末梢血中に移行した後、骨髄へホーミングすると考えられている¹⁵⁾。そこでZhanらは扁桃内に存在するCD19陽性Bリンパ球(7例)、扁桃のCD138陽性形質細胞(11例)および骨髄のCD138陽性形質細胞(31例)を、それぞれCD19あるいはCD138に対する親和性磁気ビーズカラムにて純化し、Affymetrix HuGeneFLまたはHGU95Av2にて遺伝子発現プロファイル解析を行った¹⁶⁾。その結果、これまで知られているとおりCD45, CD19, CD20等の表面マーカーは形質細胞への分化に伴い発現が低下し、一方CD138, CD38, CD63な

どは最終分化に伴って誘導されることが確認された。

Zhanらは次に分化の初期過程である扁桃内でのCD19陽性Bリンパ球からCD138陽性形質細胞への分化に伴って発現量が変化する遺伝子を抽出し、表3に示されるようにBMI1やIRF4などの転写因子、アポトーシス調節因子であるCASP10、細胞接着に関わるITGA6, PECAM1等が分化に伴って発現が誘導されることを示した。一方、分化に伴い発現が抑制される遺伝子として転写因子であるETS1等が認められた。また、その他にも細胞周期調節因子やケモカイン、接着因子など様々な遺伝子の発現が扁桃内でのBリンパ球の成熟に際し大きく変化することも示された。興味深いことに細胞増殖に直結する転写因子であるMYCは、扁桃内でのCD138陽性への分化に伴い一旦発現が減少するが、骨髄へのホーミングに際して再び発現誘導されることも明らかになった。

次に扁桃内形質細胞から骨髄の形質細胞への移行に伴い発現が制御される遺伝子であるが、表3にあるBMI1やSTCHなど扁桃内で発現が誘導された遺伝子の一部は、骨髄への移行に際し発現がさらに活性化されることも確認された。また扁桃内では全く発現がないが骨髄において初めて発現が確認された遺伝子として、表4にあるように複

表3 扁桃内で発現量が変化する遺伝子¹⁶⁾

accession no.	symbol	function	TBC	TPC	BPC
U60519	<i>CASP10</i>	apoptosis	-	+	++
X53586	<i>ITGA6</i>	adhesion	-	+	++
U04735	<i>STCH</i>	chaperone	+	++	++
L13689	<i>BMI1</i>	transcription; repressor; PcG	+	++	+++
L34657	<i>PECAM1</i>	adhesion	+	++	+++
U52682	<i>IRF4</i>	transcription; IRF family	+	+++	+++
M31627	<i>XBPI</i>	transcription; bZip family	+++	++++	++++
AB000410	<i>OGG1</i>	DNA glycosylase	+	-	-
D87432	<i>SLC7A6</i>	solute transporter	+	-	-
J04101	<i>ETS1</i>	transcription; ets family	+	-	-

TBC: tonsil B cell, TPC: tonsil plasma cell, BPC: bone marrow plasma cell

数のシグナル伝達分子が認められた。

このようにしてBリンパ系細胞の最終分化における遺伝子発現プロファイルの変化が明らかにされたが、Zhanらによる本研究の重要性はむしろ、すでに明らかにされたMMのサブタイプがこれら異なった分化段階のそれぞれに相当することを示した点であろう。彼らはまず扁桃内で発現変化する遺伝子群（表3を含む）の中で、扁桃Bリンパ球およびMM細胞間で発現量が似ているのを選びだした。また扁桃形質細胞-骨髓形質細胞間で発現変化する遺伝子群（表4）の中でMM細胞と発現量が似ているものも抽出した。これら扁桃Bリンパ球-扁桃形質細胞-骨髓形質細胞のいずれかの段階とMM細胞間で発現量が似ている遺伝子群を用いてサンプル全体の系統樹を作成したところ、形質細胞の各分化段階のサンプルと同じグループに分類される患者サンプルが明らかになった。

本データはMM細胞の起源自体が複数の分化段階の細胞から生じうることを示唆するのもかも知

れないし、少なくとも遺伝子発現様式の面からは悪性化した骨髓腫細胞が未熟な扁桃Bリンパ球に似ているものから、成熟した骨髓形質細胞に似ているものまで多様であることが明らかである。これまでMMの起源を議論するときにMM自体は（少なくとも分化段階においては）均質なものと仮定されていたが、現実は異なるようである。しかも興味深いことに、これら「異なった分化段階の形質細胞」に類似した各患者グループは、Zhanらが以前報告したMMのサブグループ（MM1～4）にオーバーラップするのである（表5）。たとえば扁桃Bリンパ球に似た患者グループ22例の内、13例が以前報告されたMM4群に属し、残りはMM1群3例、MM2群1例、MM3群4例となった。同様に扁桃形質細胞に似た患者グループ29例の内14例はMM3群に属し、骨髓形質細胞に似た患者サンプル20例の内14例はMM2群に属する。もちろん両基準による患者分類は完全に一致しているわけではなく、どちらの分類法がより正確に患者予後に相関しているかは今後の解

表4 最終分化に伴い発現量に変化する遺伝子¹⁶⁾

accession no.	symbol	function	TPC	BPC
U32114	<i>CAV2</i>	signaling; membrane caveolae	-	+
U60115	<i>FHL1</i>	signaling; LIM domain	-	+
U73936	<i>JAG1</i>	signaling; Notch ligand	-	+
X57025	<i>IGF1</i>	signaling; growth factor	-	+
Z32684	<i>XK</i>	membrane transport	-	+
D10511	<i>ACAT1</i>	metabolism; ketone	-	++
Y08999	<i>ARPC1A</i>	actin polymerization	-	++
M14745	<i>BCL2</i>	signaling; antiapoptosis	-	++
M24486	<i>P4HA1</i>	collagen synthesis	-	++
M60315	<i>BMP6</i>	signaling; TGF family	-	++

TPC: tonsil plasma cell, BPC: bone marrow plasma cell

表5 正常細胞における分化レベルに近似したMMサブグループ¹⁶⁾

分化様式でのグループ	MM1 (n=20)	MM2 (n=21)	MM3 (n=15)	MM4 (n=18)	P
扁桃Bリンパ球に近似 (n=22)	3	1	5	13	0.00005
扁桃形質細胞に近似 (n=29)	8	4	14	3	0.000008
骨髓形質細胞に近似 (n=20)	6	14	0	0	0.000001

析を待たなくてはならない。しかし、おそらく予後不良群と考えられるMM4が正常形質細胞の分化段階においては未熟な扁桃Bリンパ球に近似しており、予後良好が予想されるMM2群が最も成熟した骨髄形質細胞に似ていることは、形質細胞の悪性化機構を考える上できわめて興味深いといえる。

むすび

著者らは以前より、疾患責任分画を純化した上でゲノミクス解析を行うアプローチ（BAMPスクリーニング）の重要性を提唱してきた¹⁷⁻¹⁹。たとえば急性骨髄性白血病の解析であればCD133陽性造血幹細胞相当分画が、NK細胞増多症の解析であればCD3陰性CD56陽性NK細胞分画がそのような解析の標的として適切であろうし、MM/MGUSの解析では本稿に示すようにCD138陽性分画こそが解析対象として最適であろう。これらの解析の結果得られたMMに関する情報はいずれも貴重であるが、実際の疾患発症機構解明にどの程度寄与できるかはいまだ不明である。言い換えればチップ解析の結果得られた疾患関連遺伝子の内どれがMM発症に直接寄与しているのかは今後の分子生物学的解析を待たなくてはならない。しかしながら、疾患の診断・分類を考える上では本稿で述べたような遺伝子発現情報は貴重であり、今後は実際の患者予後、化学療法に対する反応性などの臨床情報をどれだけ精度よく予測可能かに研究の焦点が移行するであろう。また臨床応用を目指す上では、解析に必要な遺伝子数をさらに絞り込むことも重要となると考えられる。

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