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厚生労働科学研究費補助金
ヒトゲノム・再生医療等研究事業

骨髄異形成症候群の原因遺伝子の同定と発症機構の解明

平成 16 年度　総括研究報告書

主任研究者　小川　誠司

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Ⅰ．　総括研究報告書

厚生労働科学研究費補助金（ヒトゲノム・再生医療等研究事業）
総括研究報告書

骨髄異形成症候群の原因遺伝子の同定と発症機構の解明

主任研究者　小川　誠司　東京大学医学部附属病院　造血再生医療寄付講座　客員助教授

研究要旨
MDSで高頻度の欠失を認める7qの標的遺伝子の候補としてメチル化による不活化という観点から探索し、PFTK1およびQ9P1T7を同定した。また、3264個のBACクローンを搭載するHuman 1Mアレイを用いたアレイCGHシステム、ならびに116,204個の高密度オリゴヌクレオチドアレイを用いた超高解像度のゲノムコピー数/LOH解析システムを開発し、MDSにおけるコピー数の解析およびゲノムワイドなLOHの解析を行うことにより、多数の共通増幅・欠失領域を同定し、複数の標的遺伝子の候補を同定した。さらに、AML1欠失マスウの解析により、AML1がTおよびBリンパ球の分化成熟に必須の機能を担うことを明らかにするとともに、MDSにおけるリンパ球の産生の異常のメカニズムを明らかにした。

分担研究者
千葉　滋
東京大学医学部附属病院
無菌治療部　助教授
黒川　峰夫
東京大学医学部附属病院
血液・腫瘍内科　講師

A. 研究目的

骨髄異形成症候群（MDS）は骨髄不全と前白血病状態を特徴とする難治性造血器疾患であって、造血幹細胞移植を除いて有効な根治的治療手段は知られていない。一方、造血幹細胞移植は治療関連毒性が高く、高齢者に多発する本症に対する主要な治療法とはなりえない。従って、高齢者にも適応可能な治療毒性の少ない効果的な治療法の開発が急務であるが、そのためには、MDSの原因となる遺伝子（分子）の異常を同定し、当該分子に対して特異的に作用する薬剤を開発することが必要である。本研究事業の目的は、先進のゲノム解析技術を駆使して、MDSの原因となる遺伝子変異を同定し、その病態を明らかにすることである。これまでの研究においては、MDSに特異的に認められる染色体異常に伴うゲノムの異常の解析およびMDSにおけるDNAのメチル化の標的遺伝子の探索を進めてきたが、本年度は、メチル化の標的遺伝子の解析をさらに進めるとともに、昨年度より開発を進めてきたBACアレイCGH法、および今年度に新たに開発した高密度オリゴヌクレオチドアレイを用いたMDSゲノムにおけるコピー数変化の網羅的探索によるMDSの原因遺伝子の同定を試みた。加えて、昨年度までにMDSの動物モデルとして作成したAML1欠失マウスを用いてその造血系の異常をさらに詳細に解析した。

B. 研究方法

1）7番染色体のメチル化領域の標的遺伝子の同定（千葉）

MDSで高頻度に欠失する7q-における責任遺伝子を残存アレルのメチル化による不活化という観点から、同定することを目的として、昨年度までに造血器腫瘍で特異的にメチル化をうける7qの遺伝子として、25個の遺伝子を同定した。そこで本年度は、これらの腫瘍において、定量PCR法を用いた遺伝子発現の定量的解析を行い、これらの遺伝子のプロモーターのメチル化にともない発現の低下する遺伝子の同定を行った。

2) MDSにおけるゲノムワイドなコピー数の解析（小川）
相互転座型の異常の多い白血病とは対照的にMDSでは染色体の増幅・欠失を伴う異常の頻度が高いことが特徴である。そこで我々は3264個のBACプローブを搭載するHuman 1Mアレイを構築し、これを用いたアレイCGH法を用いて、62例のMDSについてゲノムに生ずるコピー数の変化を1Mbの平均解像度で網羅的に探索した。さらに、近年大規模SNP解析を目的としてAffymetrix社により開発された高密度オリゴヌクレオチドアレイを用いて、ゲノムコピー数を高精度に分析できるソフトウェアをAffymetrix社と共同開発し、これを用いたMDSのゲノム解析を行った。

3) AML1遺伝子欠失マウスの作成と病態解析（黒川）
AML1遺伝子はMDSの10-25%で不活化を認めるMDSの重要な標的遺伝子の一つであるが、我々は昨年度に、成体造血で特異的にAML1を欠失するマウスを作成し、同マウスがMDS類似の病態を呈することを示すとともに、成体型造血の確立に必須であるAML1遺伝子が成体における造血幹細胞の維持には必要ではないこと、およびこれらのマウスでは血小板およびリンパ球産生の低下が認められることを報告した。本年度は、リンパ球の産生異常に焦点をあてて、AML1を欠失した造血幹細胞に由来するリンパ球分化の異常をOP9/Detal上でのT細胞の分化のアッセイおよびB細胞前駆体における分化の異常を解析した。

（倫理面への配慮）
MDS患者試料を用いたゲノム解析については、ヒトゲノム遺伝子解析研究に関する倫理指針、疫学研究に関する倫理指針および臨床研究に関する倫理指針に基づき、東京大学医学部の設置する倫理委員会の承認を得て行った。
実験に使用したマウスの安楽死には頸椎脱臼を用い、可能な限り苦痛を与えないよう配慮した。

C. 研究結果
1) 7番染色体のメチル化領域の網羅的解析（千葉）
昨年度に報告した25個の遺伝子について、発現量を定量PCR法により測定し、各プロモーターのメチル化の状態との相関を解析したところ、メチル化レベルと遺伝子のsilencingが高い相関を示す遺伝子として、PFTK1およびQ9P1T7を同定した。これらの遺伝子は、7qで不活化をうける標的遺伝子の有力な候補と考えられた。

2) MDSにおけるゲノムワイドなコピー数の解析（小川）
我々が独自に作成したHuman 1Mアレイを用いたCGHシステムは、ヒトゲノム全域に渡って1Mbの平均解像度でコピー数の変化を鋭敏に検出することが可能で、本システムを用いて62例のMDS試料についてコピー数の変化を解析した。本解析により、従来の染色体分析では検出不可能であった微細なゲノムの増幅・欠失が多数同定された。標的遺伝子の同定という観点からは、より解析の分解能を向上させて、異常の範囲を正確にdefineすることが重要である。そこで、研究事業の最終年度には、Affymetrix社よりreleaseされている大規模SNPタイピング用に開発された高密度オリゴヌクレオチドアレイを用いたコピー数の解析システムを同社と共同開発し、これを用いてMDSにおけるゲノムのコピー数変化をさらに詳細に解析した。同アレイを用いた我々の解析システムでは、116,204個のSNPプローブを用いて、全ゲノムで平均23.6kbの分解能でゲノムに生ずるコピー数の変化を解析できること、また患者の正常組織とペアで解析することにより、アレル別にコピー数の増減が検出できること、また、これまでに前例のない解像度でゲノムワイドなLOHのマッピングを行うことができることが特徴である。解析の結果、アレイCGHでは検出できなかったいくつかのホモ接合性欠失を示す領域が同定され、これらの領域からMDSの発症に関わることが推定されるいくつかの遺伝子を見いだした。また、染色体分析では一見正常な核型を示す症例においても、相同組み換えによってuniparental disomyを広範に生じている症例が多数同定され、MDSではゲノムのremodelingが通常の解析で認められる以上に広範に生じていることが明らかとなった。こうした'UPD phenotype'は新たなゲノム不安定性の指標となるものであって、これが生ずるメカニズムを明らかにすることは、今後の課題である。

3) AML1遺伝子欠失マウスの作成と病態解析（黒川）
AML1を成体造血系で欠失させたマウスでは、末梢リンパ球の減少および血小板の減少が観察される。我々はAML1欠失マウスにおけるリンパ球の異常についてT細胞のOP9/Delta1培養系およびFACS解析を行うことにより、AML1欠失マウスではdouble negative T細胞分画におけるDN2からDN3への移行の障害、B細胞分画におけるHardy分画AからBへの移行の障害が認められ

ることを明らかにした。一方、造血幹細胞分画においては減少が認められず、以上の結果より、AML1は成体造血幹細胞の維持には必要ないが、その正常な分化には必須であることが明らかとなった。

D. 考察

1. 7q で造血器腫瘍特異的なメチル化により発現の低下を示す遺伝子としてPFTK1およびQ9P1T7が同定された。これらは7qの標的遺伝子の有力候補と考えられる。後者についてはその機能は明らかではないが、PFTK1はCdc2に相同性を有するキナーゼで神経細胞の分化に関与することが推定されており、造血細胞の分化の障害がMDSの重要な病態であることを考えると興味深い。一方、MDSへの関与を明らかにするためには、今後、さらに同遺伝子の変異解析などによる腫瘍特異的体細胞変異の探索を行うとともに、同遺伝子の機能的不活化と造血系の分化の障害の関連性の有無を検討する必要がある。

2. MDSにおけるゲノムコピー数の網羅的探索では、多数の高度増幅領域ならびにホモ接合性欠失が同定された。Swhawmee (11p15)、Q8NBE4 (7q31)、AK130123 (8p21)、gana (11q23)、flyter (22q12)、CNTNAP2 (7q35)などは複数の症例で欠失を認めるいくつかの例であるが、これらはMDSの標的遺伝子となっている可能性が示唆され、今後分子標的療法の可能性も含めて機能的な解析が必要である。

3. 我々が開発した高密度オリゴヌクレオチドアレイを用いたコピー数の解析システムでは、ゲノムの増幅・欠失を平均23.6kbの解像度、数10bpの分解能を有しており、現時点では少なくとも最も分析能力の高いコピー数解析システムである。同システムはMDSの解析のみならず、全てのヒト悪性腫瘍のゲノムコピー数の変化の解析に用いることができる。現在多くの研究室と共同研究によりヒト悪性腫瘍の網羅的な標的遺伝子の同定プロジェクトが進行中である。

4. MDSの30%の症例では、コピー数の変化を伴うことなくLOHが観察されるという現象が確認された。こうした領域はUPDと呼ばれるが、MDSで広範にUPDが生じていることは本研究で初めて明らかになったことの一つである。これは腫瘍ゲノムには我々が従来想像している以上に不安定性が存在し、remodelingを生じていることを示すもので、今後その意義の解明を行う必要があろう。

5. AML1欠失マウスでは、リンパ球の産生が強く抑制される。これは免疫系の構築を考える上で極めて重要な知見であるばかりでなく、MDSが造血幹細胞の異常でありながら、リンパ球系へのMDSクローンの関与がないというMDSの病態をよく説明するメカニズムであると思われる。AML1におけるリンパ球の分化の制御は、B前駆細胞においてはHardyのA亜分画で、またT前駆細胞においてはDN2およびDN3で生ずるが、AML1が制御していると考えられる下流の標的分子の同定が今後重要な課題である。

E. 結論

1) 造血器腫瘍における7qの網羅的なメチル化マッピングにより、7qで不活化を受ける標的遺伝子の候補として、PFTK1およびQ9P1T7を同定した。

2) 高密度オリゴヌクレオチドアレイを用いた超高解像度のコピー数変化の解析システムを開発し、これを用いたMDSゲノムの解析から、MDSで増幅・欠失を生ずる多数のゲノム領域とその標的遺伝子の候補の同定を行った。

3) AML1欠失マウスの解析により、AML1によるリンパ球分化の制御が行われる分化段階を同定した。

F. 健康危険情報

なし。

G. 研究発表

1. 論文発表

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H. 知的財産権の出願・登録状況

1. 二重カラーFISH 法による t(1;7)(q10;p10) の分子診断法の開発 (申請予定)。

2. Affymetrix GeneChip を用いた悪性腫瘍における網羅的なゲノムコピー数/LOH 解析プログラム (CNAG) の開発 (申請予定)。

Ⅱ.　研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

書籍

書者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
Ogawa S.	Molecular genetics of Myelodysplastic syndrome.		Educational Program Book: Japanese Society of Hematology and Japanese Society of Clinical Hematology	Japanese Society of Hematology and Japanese Society of Clinical Hematology	Japan (Kyoto)	2004	10-25

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Hosoya N, Qiao Y, Hangaishi A, Wang L, Nannya Y, Sanada M, Kurokawa M, Chiba S, Hirai H, Ogawa S.	Identification of a SRC-like tyrosine kinase gene, FRK, fused with ETV6 in a patient with acute myelogenous leukemia carrying a t(6;12)(q21;p13) translocation.	Genes, Chromosomes & Cancer	42	269-279	2005
Kunisato A, Ogawa S, Chiba S.	Dominant-negative activity of stem cell leukemia (SCL) lacking bHLH domain.	Blood	105	1365-1366	2005
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Ichikawa M, Asai T, Chiba S, Kurokawa M, Ogawa S.	Runx1/AML-1 ranks as a master regulator of adult hematopoiesis.	Cell Cycle	3	722-724	2004
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Kawazu M, Kanda Y, Nannya Y, Aoki K, Kurokawa M, Chiba S, Motokura T, Hirai H, Ogawa S.	Prospective comparison of the diagnostic potential of real-time PCR, double-sandwich enzyme-linked immunosorbent assay for galactomannan, and a (1-->3)-beta-D-glucan test in weekly screening for invasive aspergillosis in patients with hematological disorders.	Journal of Clinical Microbiology	42	2733-2741	2004

Ⅲ. 研究成果の刊行物・別刷

Molecular genetics of myelodysplastic syndrome

Seishi Ogawa

Department of Regeneration Medicine for Hematopoiesis,

Graduate School of Medicine, University of Tokyo

Myelodysplastic syndrome (MDS) is a constellation of bone marrow failure states characterized by a clonal expansion of abnormal hematopoietic progenitors, ineffective hematopoiesis, and frequent progression to acute myeloid leukemia (AML). Although pathogenesis of this intractable and mostly incurable disorder still remains to be fully understood, significant progress has been made to characterize molecular or genetic abnormalities that explain pathogenesis of this syndrome, including mutations of *N-RAS, p53, AML1*, and other genes, translocations involving *MLL, Evi1*, and *TEL/ETV6* genes, and abnormally methylated *p15* gene. This review provides an overview of recent advances in the field of molecular genetics of the pathogenesis of MDS with its focus on genetic abnormalities.

Introduction

Myelodysplastic syndrome (MDS) is a group of clonal myeloid disorders introduced into the FAB classification system to reorganize and reclassify 'refractory anemias' showing varying degrees of cytopenia with morphologic abnormalities in multiple blood components and a high predisposition to acute mycloid leukemia (AML)[1], although it has been criticized that MDS contains heterogeneous groups of disorders having different clinical courses and pathogenesis[2]. In addition, it shows apparent overlaps with other bone marrow failure states, including aplastic anemia, paroxysmal nocturnal hemoglobinuria (PNH), and myeloproliferative disorders (MPDs) in terms of responses to immunosuppressive therapies, appearance of PNH-positive blood cells, their clonality and cytogenetic profiles, and natural disease courses[3]. In boundary cases, therefore, some confusion may arise in differential diagnosis of these entities. In spite of these, however, common features of this syndrome are still evident in typical cases and consist of failure to produce mature blood components, clonal hematopoiesis, and a predisposition to AML, and currently it has been well established that a number of genetic alterations are involved in development of MDS. Although several other mechanisms have been also implicated in the pathogenesis of MDS, the primary roles of genetic alterations in this neoplastic disease should be underscored. In this review major findings on these genetic abnormalities in MDS in recent years will be summarized.

Clonality of MDS

Clonal hematopoiesis in MDS patients has been repeatedly tested using different methodologies[4-7,8]. Cytogenetic abnormalities are simple and reliable makers for evaluating clonality, but they may represent only specific subclones when it comes to multilineage contributions of the original MDS clone. Thus early studies on clonality in MDS by Fialkow et al., based on the hypothesis of random inacticvation of X chromosomes, employed the polymorphism of G6PD protein in women genetically heterozygous for this locus[5], and more recently, the polymorphism of the androgen receptor gene has been utilized for analyzing much larger numbers of women[6,9], although several caveats should be still in mind[10]. With these methodologies, the clonal origins of granulocytes and erythroid progenitors have been unequivocally demonstrated for MDS. On the other hand, the clonal origin of lymphocytes remains controversial, where B lymphocytes are variably involved and T lymphocytes are usually not affected in MDS[11]. This may represent the fact that MDS is originated exclusively from a myeloid precursor or alternatively, the disease involves a pluripotent stem cell and makes it incapable of producing mature B and/or T lymphocytes. It is of special note, in view of therapeutic

Address correspondence to S. Ogawa, Department of Regeneration Medicine for Hematopoiesis, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. Tel: +81-3-3815-5411; Fax: +81-3-5804-6261; E-mail: sogawa-tky@umin.ac.jp

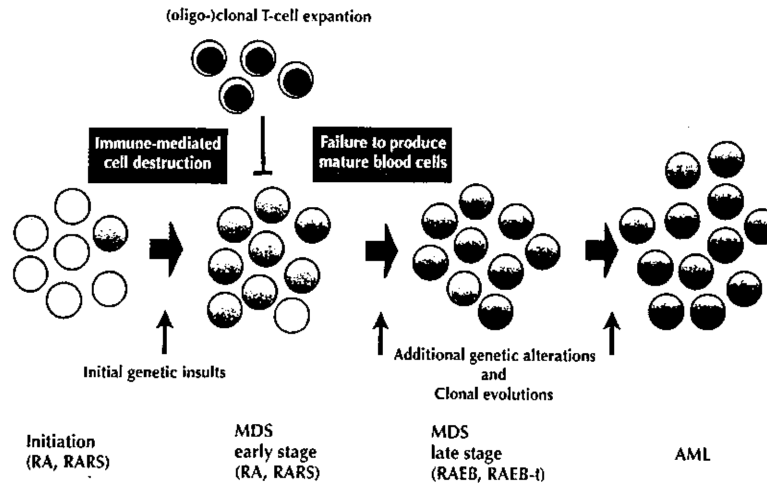

Figure 1. Multistep model of MDS pathogenesis

and pathophysiologic implications, that significant residual normal hematopoiesis could be still detected in many MDS bone marrows[12].

Multistep model of development and progression of MDS

A multistep model of genetic alterations has been frequently employed to explain the pathogenesis and development of many neoplastic disorders, including MDS. It is mainly based on temporal profiles of cytogenetic as well as other genetic abnormalities during courses of MDS, which often show emergence of new subclones having additional chromosomal abnormalities and later expansion of these subclones (Figure 1). However, it is not clear how many and what kind of genetic changes are required for development or transformation of MDS. There is no definitive evidence that multiple genetic insults are really required for development of MDS. In addition, although a number of genetic alterations have been reported in MDS patients as described below, the majority of the currently identified genetic abnormalities are found both in MDS and AML or preferentially observed in advanced or transformed cases of MDS. Thus it should be stressed that the early genetic alterations in MDS are mostly unknown.

Genetic abnormalities in MDS

A wide variety of genetic alterations have been described in MDS, including point mutations and generation of aberrant fusion genes associated with recurrent balanced translocations. A list of these abnormalities, not complete through is given in Table 1.

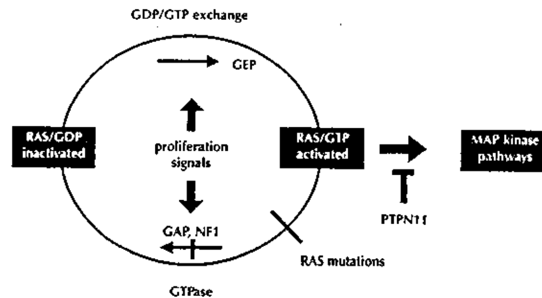

Figure 2. Abnormalities in the *Ras* pathways in *MDS*

Mutations of *RAS*, *p53*, and other genes in MDS

Among these genetic alterations, the first identified are mutations of the *N-RAS* proto-oncogene[13]. RAS is a key molecule for the MAP kinase cascade to transduce proliferation signals to nucleus. Mutations exclusively occur at codon 12, 13 or 61, which converts RAS to a constitutive active molecule (Figure 2). In contrast to early reports showing mutation rates of 30 to 40%, more recent studies reported much lower frequencies of ~10% on average[14]. *p53* is another target for mutations in MDS[15]. *p53* is the most frequently inactivated tumor suppressor gene (TSG) in human cancers and its diverse functions have been extensively studied, although the frequencies of its mutations are generally lower in hematopoietic tumors. Referring to the TP53 database, 82 of 646 (~12%) MDS cases are reported to have mutated *p53* genes (http://www.iarc.fr/p53/), but this figure may provide too high an estimation, probably suffering from a publication bias. Both *RAS* and *p53* mutations are rare in early stages

Table 1. Genetic abnormalities in MDS

Mutation, Deletion, Duplication		
N-RAS	~10%	mutation
NF1	childhood MDS, JMML	mutation
PTPN11	childhood MDS, JMML	mutation
p53	<10%	mutation, deletion
FLT3	~5%	tandem duplication
FMS	rare	mutation
KIT	rare	mutation
AML1	~10%	mutation
ATRX	rare AT-MDS	mutation
TERC	rare	mutation
Cytochrome c	rare	mutation
WT1	rare	mutation
Gene Rearrangements		
EVI1(3q26) and MEL1(1p36)		
t(3;3)(q21;q26)	*EVI1 overexpression*	
inv(3)(q21q26)	*EVI1 overexpression*	
t(3;21)(q26;q22)	*AML1/EVI1*	
t(3;12)(q26;p13),	*TEL/EVI1*	
t(1;3)(p36;q21)	*MEL1 overexpression*	
TEL/ETV6(12p13)		
t(5;12)(q33;p13)	*TEL/PDGFR-b*	
t(9;12)(q22;p12)	*TEL/SYK*	
t(1;12)(36.1;p13)	*TEL/MDS2*	
t(5;12)(q31;p13)	*ASC2/TEL*	
t(12;22)(p13;q11)	*MN1/TEL*	
MLL(11q23)		
t(11;16)(q23;p13)	*MLL/CBP*	
t(11;19)(q23;p13.1)	*MLL/MEN*	
t(5;11)(q31;q23)	*MLL/GRAF*	
NUP98 and CAN		
t(7;11)(p15;p15)	*NUP98/HOXA9*	
inv(11)(p15q22)	*NUP98/DDX10*	
t(2;11)(q31;p15)	*NUP98/HOXD13*	
t(11;17)(p15;q21)	*NUP98/HOXB*	
t(11;12)(p15;q13)	*NUP98/HOXC13*	
t(11;20)(p15;q11)	*NUP98/TOP1*	
t(6;9)(p23;q34)	*DEK/CAN*	
Other		
t(3;5)(q25;q34)	*NPM/MLF-1*	

of MDS (RA/RARS) and typically found in more advanced stages (RAEB/RAEBt) or during transformation to AML[16].

Activated mutations of receptor tyrosine kinases have been also reported in MDS, including mutations of the *c-FMS* gene encoding M-CSF receptor and of the *FLT3* gene[17-19]. Mutations of the *c-FMS* gene were reported to be more common in CMMoL but have not repeatedly confirmed, while the *FLT3* mutations are considered more important for the pathogenesis of AML or progression form MDS to AML rather than development of MDS itself. Mutations of the *Chk2* gene, and human telomerase RNA gene (*TERC*)[20] and deletion of α-*globin* gene clusters[21] are also found in isolated reports. Inactivation mutations of the *ATRX* gene are found in rare cases of AT-MDS characterized by myelodysplasia with severe microcytic anemia due to α-thalathmia[22]. ATRX is a SWI/SNF like protein and involved in transcriptional regulation of genes including the α-globing gene. Constitutional inactivation of the *ATRX* gene causes ATRX syndrome, a rare X-linked disorder showing a-thalathmia, mental retardation, facial dysmorphism, and urogenital abnormalities[23,24]. Since patients with ATRX syndrome do not develop MDS, acquired *ATRX* mutations in AT-MDS cases are not considered to play a role in initiation of MDS, but to modify its phenotype. Mutations in mitochondrial respiratory genes such as the *cytochrome c oxidase (CXO)* gene have been also reported in MDS patients in high frequencies[25]. Since defects in CXO will compromise sufficient oxidative energy production in mitochondria required for the driving force of mitotic spindles, it raises an attractive hypothesis that inactivation of the *CXO* gene will lead to genetic instability due to mitotic dysfunction[25], but the observation has not been firmly confirmed[26].

AML1/Runx1 gene

AML1 or *Runx1* is a well-known target of t(8;21)(q22;q22) translocation found in AML, in which *AML1* is rearranged with *ETO* to generate the *AML1/ETO* fusion gene[27]. *AML1* is also involved in t(12;21)(p13;q22) and t(3;21)(q26,q22) to form TEL/AML1 and AML1/Evi1 fusion protein[28,29], respectively. It encodes a transcription factor that regulates transcription of a wide variety of genes expressed in hematopoietic compartments and is indispensable for establishment of definitive hematopoiesis[30-32]. Importantly, a germ line mutation of *AML1* causes a rare hereditary disorder known as the familial platelet disorder with a predisposition to AML (FPD/AML)[33]. It typically has several years of a preleukemic period showing dysmegakaryopoiesis with reduced platelet counts and may be considered as a kind of congenital form of MDS, in which an *AML1* mutation is clearly the first and possibly sufficient genetic hit that contributes to its MDS-like phenotype. Of particular note is that acquired mutations of *AML1* are commonly found in sporadic cases of AML (53/619; 8.6%) especially of M0 phenotype (39/185; 21%), and also in MDS cases

(33/362; 9.1%) (Figure 3)[34-38]. An excellent review is available covering both sporadic and familial mutations of *AML1*[39]. *AML1* mutations may be hemi- or biallelic with a substantially higher biallelic mutation rate in the AML M0 subtype[34,38,40]. In MDS *AML1* mutations seems to be detected in more advanced stages (RAEB/RAEBt and MDS derived from AML) and in therapy related MDS (tMDS)[41]. Recently a strong association of *AML1* mutations with monosomy 7 has been reported in advanced stage MDS, suggesting cooperative roles between both abnormalities[37]. *AML1* mutations can be rarely detected in apparently healthy persons and frequently found in atomic bomb-related MDS cases. Thus *AML1* locus may be more prone to mutations[40,42,39]. Some mutations lead to simple loss-of-function proteins, while others clearly generate mutants having dominant-negative activities against the normal AML1 protein. Propensity to AML also differs among FPD/AML pedigrees and possibly among sporadic cases with different *AML1* mutations, depending on types of the mutations[33,43].

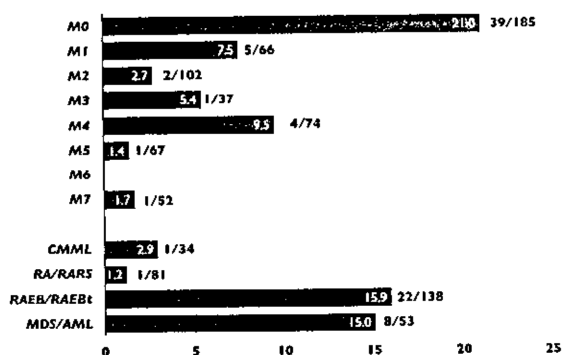

Figure 3. Frequencies of *AML1* mutations

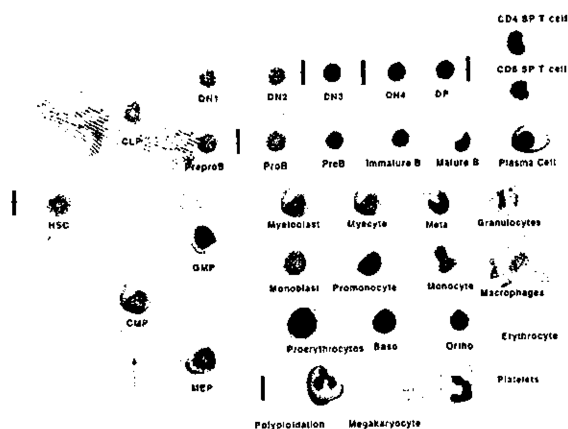

Figure 4. Loss of *AML1* blocks normal hematopoiesis at multiple stages

Several mouse models carrying abnormal *AML1* genes have been generated. *AML1*-null mice are embryonic lethal on E12.5 and *AML1/ETO*-knockin mice also show a similar phenotype, indicating that AML1-fusion protein act in a dominant negative manner[44,45,46]. Unexpectedly, however, when *AML1* is disrupted in adult bone marrow, hematopoietic stem cells are still maintained or rather increased in number, while severe dysmegakaryopoiesis prevails showing defects in polyploidation and reduced platelet counts. Intriguingly, production of both mature T cells and B cells is also severely impaired in the *AML1-null* mice (Figure 4)[47], which may give rise to a plausible explanation for frequent sparing of lymphoid lineages in MDS.

NF1 gene

The *NF1* gene was originally identified as the causative gene for neurofibromatosis type I (NF1) and encode neurofibromin, a GTPase-activation protein (GAP) for p21RAS[48,49]. *NF1* is one of the prototypes of a tumor suppressor gene, inactivation of which leads to constitutive activation of p21RAS (Figure 2)[50]. NF1 patients has a strong predisposition to developing Juvenile Myelomonocytic Leukemia (JMML) (~200 to 500 fold increase in relative risk to the normal population), an aggressive form of childhood MDS characterized by monocytosis, thrombocytopenia, splenomegaly, and malignant infiltration of the skin, lymph nodes, lungs, liver, and other organs. In a large series of JMML, 14% were found to have NF1[51]. In mouse models somatic inactivation of *NF1* in hematopoietic cells results in a progressive myeloproliferative disorder resembling JMML[52]. It should be noted that *NF1* is mutated and inactivated in sporadic cases of childhood MDS or JMML, especially in combination with monosomy 7[53], although *NF1* mutations is rare in adult cases. Oncogenic *RAS* mutations were also found in JMML in high frequency and restricted to cases without NF1, underscoring pathogenic importance of the RAS-activating pathway for the pathogenesis of JMML[54].

PTPN11 gene

PTPN11 encodes SHP-2 protein tyrosine phospatase and is congenitally mutated in Noonan syndrome[55], a developmental disorder with short stature, facial dysmorphia,

skeletal anomalies, and occasional development of JMML. Intriguingly, somatic mutations of the *PTPN11* gene were found in 10 of 62(16%) sporadic cases of JMML, 5 of 50 (10%) other childhood MDS cases, and 1 of 26 (3%) AML[56,57]. SHP-2 seems to act in the RAS/MAPK cascade, because mutant SHP-2-introduced cells showed sustained activation of ERK2 in response to EGF stimulation (Figure 2)[58]. Also supporting this is that mutations were not detected in JMML associated with NF1 cases and in those who had *RAS* mutations. Mutations occurred exclusively in childhood MDS with advanced diseases as well as JMML[57]. No *PTPN11* mutations have been reported in adult MDS[59].

Cytogenetic abnormalities in MDS

A huge number of cytogenetic abnormalities have been described in MDS and provide important clues to delineate molecular bases for MDS pathogenesis[60]. Frequent cytogenetic abnormalities found in MDS are listed in Table 2. Although many of these abnormalities are common to both MDS and AML, rarity of disease-specific balanced translocations and high frequencies of unbalanced abnormalities compared to *de novo* AML, are among prominent cytogenetic features of MDS. tMDS tends to have more complex chromosomal anomalies, higher rates of hypodiploid, 5q-/-5, and 7q-/-7, and lower frequencies of trisomy 8 and hyperdiploid than *de novo* MDS[61].

Among recurrent unbalanced abnormalities in MDS are trisomy 8, 7q-/-7, 5q-/-5, 20q-, 13q-, 12p-, and 17p-[62]. A predominance of loss of, rather than gain of genetic materials may indicate importance of inactivation of TSGs for the pathogenesis of MDS[61]. However, in spite of a great deal of effort in the field of molecular genetics, no relevant TSGs has been successfully identified within the recurrently deleted chromosomal segments, while a number

Table 2. Common cytogenetic abnormalities in MDS/tMDS

30-50% of primary and ~80% of secondary MDS have chromosomal abnormalities.

Numerical	Translocations	Deletions
+8 (~20%)	inv3 & t(3;3) (4%)	del 5q (~25%)
-7 (~15%)	t(1;7) (2%)	del 11q (~3%)
-5 (~7%)	t(1;3) (1%)	del 12q (~5%)
-Y (~8%)	t(6;9) (<1%)	del 20q (~6%)
-17 (~4%)	t(5;12) (<1%)	del 7q (~5%)
		del 17p (~4%)
		del 13q (2%)

of target genes were identified from the breakpoints analysis of recurrent reciprocal translocations found in MDS/AML. In addition to technical difficulties arising from a large size of involved chromosomal segments to be analyzed, possibilities of haploinsufficiency and multiple target genes with regard to MDS pathogenesis may complicate molecular analysis of putative TSGs from chromosomal deletions.

Translocations

As mentioned above, balanced translocations are more characteristic features of *de novo* AML than of primary MDS. However, they clearly play important roles in progression of MDS into AML. *Evi1* and *MEL1*, *MLL*, and *TEL/ETV6* are among genes most frequently involved in these translocations.

3q21q26 syndrome and t(1;3)(p36.1;q21)

t(3;3)(q21;q26) and inv(3)(q21q26) are observed in ~2% and ~4% of MDS, respectively, and also found in a similar proportion of AML cases. Many of these cases have common features of multilineage dysplasia, a normal to elevated platelet count with increased dysplastic megakaryocytes, minimal or no response to chemotherapy, and poor prognosis[63]. The former two abnormalities in AML are known as 3q21q26 syndrome, in which overexpression of *Evi1* seems to be a common finding although there is a conflicting report[64]. In 3q21q26 syndrome, it is postulated that juxtaposition of the *Evi1* gene to the *ribophorin I (RPN1)* locus on 3q21 leads to aberrant *Evi1* expression (Figure 5)[65]. *Evi-1* was originally identified at the common retrovirus integration site in myeloid leukemia from AKXD inbred mice, and encodes a transcription factor having two zinc finger motifs[66]. Later an alternative splicing form of Evi1 with an additional N-terminal sequence, referred to as *MDS1/Evi1*, was revealed to have a PR domain similar to RIZ and RPDM1 proteins, which also have an alternative isoform that lacks a PR domain (Figure 5)[67]. In 3q21q26 syndrome, a shorter Evi1 isoform is exclusively expressed from the rearranged allele, in which the *MDS1* promoter is lost or located too distant (~500kb) from the putative enhancer element of *RPN1* by gene rearrangements (Figure 5)[68].

Evi1 is also involved in other translocations and transcriptionally activated as found in t(2;3)(p15;q26), t(3;7)(q26;q22), and (3;13)((q26;q13-14)[69-71]. In addition, aberrant fusion genes involving *Evi1* have been reported

in other myeloid neoplasms especially tMDS, tAML, and myeloid crisis of CML carrying t(3;21)(q26;q22) and t(3;12)(q26;p13), in which *AML1/Evi1* and *TEL(ETV6)/Evi1* are generated, respectively[29,72]. Moreover, increased *Evi1* expression is also observed in ~9% of other AML and MDS cases as well as CML BC cases without 3q26-involving translocations or inversions and related to poor prognosis, suggesting critical roles of *Evi1* in human myeloid leukemogenesis[73-76].

Evi1 is presumed to bind specific DNA sequences and act as a strong repressor, for example, of GATA-1. In other contexts, it enhances AP1 activity[77] and can transform Rat-1 fibroblast *in vitro*. It also binds to Smad3 and inhibit TGF β signaling[78], which may be mediated by a transcriptional corepressor, CtBP[79]. Evi1 was also shown to interact with HDAC-1[80], which could mediate the repressor function of Evi1. Differential functions of MDS1/Evi1 and Evi1 isoforms are implicated in leukemogenesis[81], but their leukemogenic roles seem to be still controversial.

t(1;3)(p36;q21) is another translocations found in rare (~1%) cases of MDS and AML with similar clinicopathologic features to 3q21q26 syndrome. Interestingly, *MEL-1* on 1p36 that is highly homologous to *Evi1* is translocated to the *RPN1* locus on 3q21 and transcriptinally activated[82]. MEL1 also has two alternative splicing forms that are closely related to Evi1 and MDS1/Evi1, and a smaller *MEL1* product lacking a PR domain (MEL1S) is preferentially expressed in t(1;3)(p36;q21) (Figure 5)[83]. Similarity in disease phenotypes and involved genes seems to strongly support the idea that these '*Evi1* family genes' are the *bona fide* targets of 3q21q26 syndrome and t(1;3)(p36;q21).

TEL/ETV6 translocations

TEL or ETV6 is an ETS-like transcription factor first identified at the 12p13 breakpoint of t(5;12)(q33;p13), and reported to be essential for development and maintenance of hematopoiesis as well as for megakaryopoiesis (Blood 102:131a, 2003). In this translocation, TEL is fused to PDGFR-β (platelet-derived growth factor β), resulting in TEL/PDGFR-β fusion protein. t(5;12)(q33;p13) is associated with a rare form of CMMoL showing myelomonocytic proliferations with frequent eosinophilia[84], and may be more properly grouped together with t(1;4)(q44;q12) in hypereosinophilic syndrome, and t(4;22)(q22;q11) and t(9;12)(q34;p13) in *Ph1* negative CML variants, in which FIP1L1/ PDGFR-α, and BCR/ PDGFR-α and TEL/ABL are generated, respectively[85-87]. *TEL* assumes a promiscuous feature to generate various fusion genes with different partner genes in a wide variety of hematopoietic neoplasms including MDS and AML derived from MDS[88]. *Syk*, *MDS2*, *Evi1*, *ASC2*, and *MN1* are among fusion partners of *TEL* gene in t(9;12)(q22;p12), t(1;12)(36.1;p13), t(3;12)(q26;p13), t(5;12)(q31;p13), and t(12;22)(p13;q11), respectively[72,89-92].

TEL seems to provide an interface for dimerization via its HLH domain and to activate fused kinases in some translocations, while in others, apparently functionless,

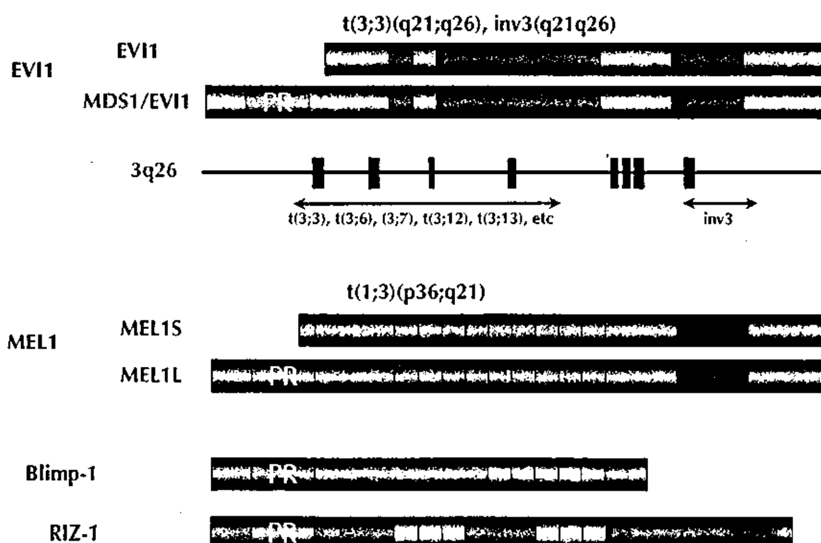

Figure 5. 1p36/3q26 Syndrome

small fusion products are translated. The *TEL* locus is involved in 12p deletions in MDS/AML, and the non-rearranged *TEL* allele seem to be inactivated in *TEL*-involving translocations[93]. These findings seem to support an idea that TEL acts as a tumor suppressor and that inactivation of TEL functions may lead to deregulated hematopoiesis and leukemogenesis.

MLL translocations

MLL or *HTRX*, a human homologue of the *Drosophila* trithorax gene, is another target of balanced translocations. It was originally identified as a fusion partner of *AF4* from the 11q23 breakpoint of t(4;11)(q21;q23) translocation, which is closely associated with infantile biphenotypic leukemia[94]. Now an increasing number of fusion genes involving *MLL* have been identified from leukemia-associated translocations, including t(9;11)(q21;q23) and t(11;19)(q23;p13.3)[95]. In view of MDS pathogenesis, t(11;16)(q23;p13) and t(11;19)(q23;p13.1), as well as t(5;11)(q31;q23), have been reported in tMDS/tAML and molecularly delineated, in which CBP and MEN, as well as GRAF are fused with MLL, respecctively[96-98]. Tandem duplication of *MLL* has been also identified in some cases of MDS[99].

MLL is presumed to participate in epigenetic gene regulation that is relevant to development and differentiation of hematopoietic cells[100]. It interacts with SNF5, a component of a SWI/SNF complex[101] and contains a SET domain showing histone methyltransferase activity, which is lost in aberrant fusion proteins[102]. Thus MLL seems to regulate gene expression by chromatin modifications, and loss of its functions may be responsible for the pathogenesis of leukemia and MDS.

Translocations involving _NUP98_ gene

11p15 is also a recurrent breakpoint found in tMDS/tAML, in which the *NUP98* gene is located. NUP98 is a nucleoporin involved in nuclear transport of protein and RNAs[103]. A number of chimeric proteins including NUP98/HOXA9, NUP98/DDX10, NUP98/HOXD13, NUP98/HOXB, NUP98/HOXC13, NUP98/TOP1 bave been reported in t(7;11)(p15;p15), inv(11)(p15q22), t(2;11)(q31;p15), t(11;17)(p15;q21), t(11;12)(p15;q13), and t(11;20)(p15;q11), respectively[104-108]. The other nucleoporin gene that participates in pathogenesis of AML/MDS is *CAN* or *Nup214*, which is fused with *DEK* to generate the *DEK/CAN* chimeric gene in t(6;9)(p23;q34), a translocation predicting a poor clinical outcome[109].

Unbalanced chromosomal abnormalities

7q-/-7 and der(1;7)(q10;p10) translocation

Monosomy 7 and a complete or partial loss of the long arm of chromosome 7 are of particular importance because these are among the most frequent cytogenetic lesions in MDS and associated with very poor prognosis[110-112]. In adults 7q-/-7 is usually seen in association with other cytogenetic abnormalities such as 5q-/-5[113], while it tends to be the sole abnormality in childhood. It may be found in *de novo* MDS (~20%), but more typically related to tMDS/tAML (~45%).

Monosomy 7 syndrome refers to a combination of monosomy 7 as the sole cytogenetic abnormality and development of myeloid neoplasms in childhood especially less than 4 years of age. Recurrent infections, hepatosplenomegaly, lymphadenopathy, defective neutrophil chemotaxis, a male predominance, and poor prognosis are among features that characterize this syndrome. Since monosomy 7 syndrome is prevalent in JMML cases, and since both monosomy 7 and JMML share many clinico-pathologic features in common, including frequent activation of the RAS pathway, there seems to be a significant overlapping between both entities[112]. Familial cases of monosomy7 and myeloid neoplasms are known, but in such cases monosomy 7 is not germline in origin, arguing that monosomy 7 is a consequence of some mutator effects from other genetic loci[114-117]. Based on chromosome banding analysis, two critical regions of 7q deletions have been delineated: one in 7q22 and the other in 7q32-q35[118-122], and detailed FISH-based analysis of 7q- has disclosed more heterogeneous groups of deletions. No TSGs have been successfully identified as a target of 7q-.

der(1;7)(q10;p10) is an unbalanced translocations found in ~2% of MDS and AML[61], especially of tMDS/tAML in association with use of alkylating agents[123-125]. Other clinical features of der(1;7)(q10;p10) include refractory cytopenia, trilineage displasia, a high propensity to leukemia, and a poor clinical outcome. Breakpoint analysis of der(1;7)(q10;p10) disclosed that the breakpoints are randomly distributed within the large clusters of centromere alphoid sequences (~0.5~3Mb), D1Z7 on chromosome 1 and D7Z1 on chromosome 7[126]. Thus no specific genes are involved in the breakpoints but loss of 7q and/or gain

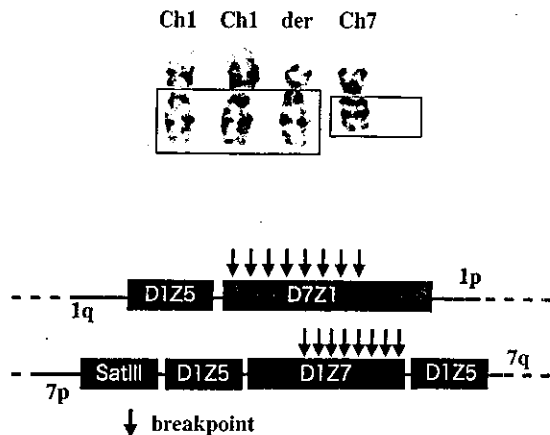

Figure 6. Partial karyotype of der(1;7)(q10;p10) and breakpoint distribution

of 1q materials resulting from the translocation should be important for the pathogenesis of MDS/AML having this translocation (Figure 6). Although it may represent a variant of 7q-, differences still exist in cytogenetic profiles between der(1;7)(q10;p10) and typical 7q-. der(1;7)(q10;p10) frequently occurs as the sole chromosomal abnormalities or in combination with one or two characteristic additional abnormalities, usually trisomy 8 and 20q-, while typical 7q- in adults usually appears as one of complex anomalies and in association with 5q-/-5, indicating der(1;7)(q10;p10) positive MDS/AML is likely to represent a distinct pathologic entity. In this regard, it is of note that 1q trisomy results from a number of similar 'centromeric unbalanced translocations' reported in MDS, including t(1;12)(q10;p10), t(1;15)(q10;p10), t(1;16)(q10 ;o10), t(1;18)(q10;p10), and t(1;21)(q10;p10), indicating roles of 1q+ in the pathogenesis of myeloid tumors[127].

5q-/-5

Abnormalities of chromosome 5 in MDS include monosomy 5, interstitial deletion of 5q and unbalanced translocations, among which interstitial deletion of 5q is most frequently observed. Especially, a combination of MDS and 5q- as the sole abnormality is referred to as 5q- syndrome and typically found in primary MDS cases[128]. It has a female predominance with a female to male ratio of 3:1 and generally shows rare leukemic transformation, very good prognosis, refractory anemia, high or normal platelet counts, and small hypolobulated megakaryocyts, which add up to a distinctive entity as found in the new WHO classification. On the other hand, 5q loss is also observed in combination with other cytogenetic abnormalities, such as 7q-/-7. Familial cases with 5q- and MDS have been also described[129].

Extent of 5q deletions is highly variable among different cases, but the critical region of 5q deletion seems to contain 5q31-q33. Of interest is that a number of cytokine-related genes, including genes for IL-3, IL-4, IL-5, M-CSF, GM-CSF, and M-CSF receptor, are clustered together and implicated in the pathogenesis of 5q- syndetome[130,131]. Other candidates of target genes for 5q deletion are *IRF-1*, *EGR-1*, and *PURA-*. IRF-1, a transactivator of interferon genes, is deleted in 90% of 5q- cases and thought to be a candidate for a relevant TSG for 5q-[132,133]. No tumor specific mutations, however, have thus far described for *IRF-1*[134]. In molecular cytogenetic approach, the smallest commonly deleted region has been currently narrowed to 1 to 1.5 Mb between D5S479 and D5S500[135].

Finally, with regard to the pathogenesis of 5q- syndrom, a recent report on a possible effectiveness of CC-5013 (REVIMID), a thalidomide analogue, may be intriguing, in which ten of eleven patients with 5q- syndrome achieved a complete transfusion independent response with REVIMID treatment and, very significantly, also resulted in disappearance of the chromosomal abnormality (5q-) in each of these patients (http://www.celgene.com/).

20q-

Deletion of 20q is most commonly found in MPD, particularly in polycythemia vera (~10%), but also described in ~5% of MDS and ~2% of AML[61]. 20q- appears solely or accompanied by other abnormalities such as 7q- and 5q-. It predicts generally favorable prognosis and, together with -Y and 5q-, is defined as a favorable cytogenetic abnormality in IPSS[136,137]. A previous report demonstrated that 20q- could not be detected in purified peripheral granulocytes in patients having 20q- in bone marrow cells despite that HUMARA assays unequivocally showed clonal granulopoiesis[138]. Thus it seems likely that 20q- may arise as a secondary event within the preexisting MDS clones, while 20q- positive clones may not contribute to mature granulopoiesis. The critical deletion spans from 20q11.2 to 20q13.2, which is now reduced to the regions between S20S17 and D20S174. Candidates of relevant TSGs include TOP1 and phospholipase C δ[139].

Other loss of chromosome materials