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「高齢者特発性造血障害の大規模ゲノミクス解析による病態解明」に関する研究

平成１６～１８年度　総合研究報告書

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総合研究報告書
「高齢者特発性造血障害の大規模ゲノミクス解析による病態解明」に関する研究

主任研究者：　間野　博行　　自治医科大学医学部　教授

研究要旨：DNA チップを用いることで数千～数万の遺伝子に関する発現変化を比較的簡便に解析することが可能となり、これまでは鑑別診断が困難であった血液疾患の診断に役立つ新たな分子マーカーが同定されると期待される。しかし DNA チップはあまりに高感度な検査法であるため、異なった造血障害患者の骨髄細胞全体を比べるような単純な解析を行うと、両患者の「骨髄中の構成細胞の違い」を反映した偽陽性結果を得ることになる。我々は広く患者さんの骨髄より造血幹細胞相当分画のみを純化し保存する「Blast Bank」を設立した。現在まで既に 800 例を越えるサンプルの保存に成功しており、本バンク細胞を用いた大規模 DNA チップ解析によって、患者の長期予後にリンクする遺伝子の同定、およびこれら遺伝子発現量に基づく予後予測法の開発に成功した。また染色体ゲノム定量を SNP タイピングアレイによって行い、造血障害特異的な染色体異常、及びそこにマップされる遺伝子の同定に施工した。またさらにプロテオミクス等によっても病態の解明につながる新規知見を得ることができた。

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A　　研究目的

骨髄異形成症候群（MDS）は赤血球を含む各種血球の慢性減少を特徴とする疾患であり、白血球減少に伴う感染症に対する治療や、赤血球・血小板の減少に対する成分輸血をしばしば必要とする。本症は末梢血中の血球減少にもかかわらず患者骨髄中の造血細胞数はむしろ正常～増加することが多く、「無効造血」と呼ばれる特徴的な病態を呈する。骨髄異形成症候群は造血幹細胞のクローン性異常に起因すると考えられているが、その具体的な分子メカニズムは未だ全く不明のままである。本疾患の年間発症率は人口 10 万人あたり 60 歳台で約 10 人、80 歳台で約 100 人と高齢化に伴い急速に上昇し、本邦における高齢者の主要な血液疾患の一つとなっている。治療法も他家骨髄移植以外に有効な方法が無く、発症時の年齢から骨髄移植の適応外であることが殆どである。さらに本疾患の一部は急性白血病へと移行する事が知られており、骨髄異形成症候群から移行した白血病の多くは薬剤耐性である。したがって今後の本邦人口のさらなる高齢化を考慮すると、骨髄異形成症候群の病態解明、診断及び治療法の開発は血液内科学に限らず今日の医学研究の急務の一つであるといえる。

ヒトゲノムプロジェクトの結果得られた遺伝子情報を元に DNA チップなどを用いた疾患解析が可能となってきた。しかし単純に患者骨髄細胞を用いて DNA チップによる比較を行った場合、各個人間の骨髄構成細胞のポピュレーションの違いが大きいため「偽陽性」な結果を得ることが殆どである。そこで真に骨髄異形成症候群の臨床にフィードバック可能な情報を得るため、我々は本研究計画において骨髄異形成症候群を含めた各種血液疾患患者より、疾患の種類によらず分化レベルがほぼ均一である造血幹細胞分画のみを大規模に収集する造血幹細胞バンク「Blast Bank」を設立する。これら純化した造血幹細胞間で DNA チップ解析及びプロテオミクス解析を行うことによって、偽陽性の極めて少ない効率的なゲノミクス解析が可能になり、世界に先駆けた病態解明が行われると期待される。本研究計画の具体的な目標として、骨髄異形成症候群の(1)分子診断、(2)発症機構の解明、(3)薬剤耐性獲得機構の解析、及び(4)新たなアプローチによる治療法の開発を目指す。

B　　研究方法

1）造血幹細胞特異的マーカーである CD133 に対するアフィニティカラムを用いて、白血病を含む各種特発性血液疾患患者骨髄より造血幹細

胞分画を純化保存し、これを Blast Bank と名付けた。平成 18 年 3 月現在で 800 例を越えるサンプルの保存に成功しており、これは純化細胞を用いたゲノミクスプロジェクトとしては世界最大級である。

2) 上記検体群を用いて以下のように DNA チップ解析を行った。細胞よりトータル RNA を抽出し、これを T7 RNA ポリメラーゼを用いてまず in vitro にて増幅した。さらにこれをもとに二本鎖 cDNA を合成し、ビオチン CTP の存在下で再び T7 RNA ポリメラーゼと反応させることで、ビオチン標識化した complementary RNA (cRNA) を作製した。このビオチン化 cRNA を DNA チップとハイブリダイズさせ、洗浄後、蛍光色素 PE 結合アビヂンと反応させた。この DNA チップ上の cRNA 結合スポットを Affymetrix 社の蛍光スキャナーで励起させ、各スポットの蛍光強度を測定した後統計処理を GeneSpring 7.0 (Silicon Genetics 社)にて行った。

3) RA 患者および正常人の末梢血よりデキストラン法を用いて好中球を分離した。その細胞より蛋白を抽出した後, 二次元電気泳動を行った。両者の泳動パターンを解析ソフト(Ettan progenesis)で解析した。発現量に差が認められたスポットの質量分析を MALDI-TOF/TOF MS を用いて行い, その蛋白についてペプチドデーターベース(MASCOT)を用いて同定した。また同定した蛋白に対する各種ポリクローナル抗体を作成し、その細胞内局在を免疫組織染色により検討し、また免疫沈降およびウエスタンブロッティングにより蛋白解析行った。さらに、好中球におけるそれらの遺伝子発現についてリアルタイム PCR 法を用いて検討した。

4) PPARγ リガンドとしては、生体内に存在する生理的リガンドの PGJ2 と合成リガンドのピオグリタゾン (製品名アクトス) を用いた。PPARγ の阻害剤としては、HX531、GW9662、BADGE を用いた。PPARγ が、そのリンガンド依存性にその核内受容体としての転写活性を発揮しているかに関しては、その特異的転写産物である adipocyte fatty acid binding protein (aP2)の転写によって確認した。細胞分化の指標としては、細胞の形態 (好中球分化による核のくびれ) と活性酸素産生能 (NBT 還元能) を評価した。PPARγ 蛋白の確認は、イムノブロットにより行った。細胞内の脂肪滴は、ナイルレッド染色により同定し、トリアシルグリセロールの定量は薄相クロマトグラフィーにより行った。

(倫理面への配慮)

検体収集に関しては自治医科大学及び栃木県立がんセンターの生命倫理委員会認可を受けた事業として開始し、連結可能匿名化のもとで研究を行った。

C　　研究結果

1)我々は平成11年8月よりBlast Bankを立ち上げ既に700例を越えるサンプルのストックに成功した。現在本バンク中に130例を超えるMDSおよび急性骨髄性白血病 (AML) サンプルが保存されており、世界的にも極めて貴重なリソースとなっている。これらBlast Bank分画を用いた解析が旧来の骨髄単核球全体を用いたものに比べ実際に偽陽性データが少ないこと、またバンクに用いるAC133陽性細胞がこれら疾患の責任クローンを含むことなども既に確認している

2) 全ヒト遺伝子が配置されたAffymetrix社GeneChipを用いた解析によってBlast Bank白血病検体における網羅的遺伝子発現定量を行った。さらに症例臨床情報と組み合わせることで、患者の長期予後に発現量がリンクする遺伝子 (予後予測遺伝子) をスクリーニングすることに成功した。これら予後予測遺伝子の発現量を基にさらに長期予後を予測するアルゴリズムを開発し、それによって実際に症例の化学療法反応性を予測可能なことが明らかになった。さらにSNPタイピング用DNAマイクロアレイを用いた患者検体ゲノムDNAの定量プロジェクトも行った。MDSおよびMDS由来白血病の骨髄細胞を用いてゲノムDNAを抽出し、Affymetrix 100K SNPタイピングアレイ、HGU133 Plus 2.0アレイおよびAgilent CGH44Aアレイそれぞれに DNAをハイブリダイズさせた。得られたデータより、MDSサンプルの微細なゲノムDNA量変化を明らかにすることができた。

3) RA 患者と正常者の末梢血好中球由来の蛋白の泳動パターンを解析ソフトで解析したところ, RA において異常発現しているスポットが認められた。それらのスポットについて MS によるペプチドマップを作製し, データベース上でペプチドマス・フィンガープリント測定を行い, 蛋白を同定した。そのうちの 2 つは CapG および Thiol-specific antioxident protein (TSA)であった。さらに RA 好中球における CapG の細胞内局在を検討するために、各種抗 CapG 抗体を作成し、それを用いて好中球の免疫染色を行い, 共焦点レーザー顕微鏡にて検討した。その結果、CapG は正常好中球ではほとんどすべての細胞において細胞質優位に存在していたのに対し、RA では核内優位に高発現する好中球が高頻度

に認められた。また、抗チロシンリン酸化 CapG 抗体を用いて解析したところ、RA 好中球においてはチロシンリン酸化 CapG が明らかに核内優位に存在し、細胞質にはほとんど発現していなかった。一方、正常好中球では細胞質内とくに細胞膜周辺に存在し、核内にはほとんど認められなかった。また、TSA の発現は RA 患者好中球において増加していたが、mRNA 発現についてリアルタイム PCR 法で検討したところ、RA と正常人の好中球の間には明らかな発現量の違いは認められず、posttranslational な機序により、増加しているものと考えられた。

4) PPARγ 蛋白が NB4 細胞に存在するか否かについてイムノブロットにより確認したところ、未分化な NB4 細胞と核内受容体リガンドで刺激した NB4 のいずれにおいても、ほぼ同量の PPARγ 蛋白が確認された。生理的リガンドの PGJ2 と合成リガンドのピオグリタゾン（製品名アクトス）のいずれも NB4 細胞の増殖を抑制し、低濃度の ATRA（RAR/RXR リガンド）はこれらの PPARγ リガンドによる増殖抑制を相加的に増強した。

D&E. 考察及び結論

本研究事業において骨髄異形成症候群の大規模な純化細胞 DNA チップ解析を行い、膨大な遺伝子発現データを得た。これらを元に「発現量から統計的有意に診断」を可能にする遺伝子群の抽出に成功し、カスタム DNA チップによる診断法の可能性を示した。またプレテオミクス技術から MDS 細胞を解析する事により蛋白質レベルでの MDS の異常を同定した。またこれら異常遺伝子・蛋白質を標的とした分子療法の開発に向けて基盤技術の開発に成功した。

F. 健康危険情報

無し

G. 研究発表

1. 論文発表

間野博行

1) Yamashita Y, Minoura K, Taya T, Fujiwara S-i, Kurashina K, Watanabe H, Choi YL, Soda M, Hatanaka H, Enomoto M, Takada S & Mano H: Analysis of chromosome copy number in leukemic cells by different microarray platforms. *Leukemia*, in press, 2007.

2) Kano Y, Akutsu M, Tsunoda S, Izumi T, Kobayashi H, Mano H & Furukawa Y: Cytotoxic effects of histone deacetylase inhibitor FK228 (depsipeptide, formally named FR901228) in combination with conventional anti-leukemia/lymphoma agents against human leukemia/lymphoma cell lines. *Invest New Drugs*, **25:** 31-40, 2007.

3) Choi YL, Kaneda R, Wada T, Fujiwara S, Soda M, Watanabe H, Kurashina K, Hatanaka H, Enomoto M, Takada S, Yamashita Y & Mano H: Identification of a constitutively active mutant of JAK3 by retroviral expression screening. *Leuk Res*, **31:** 203-209, 2007.

4) Yamashita Y, Ohashi J, Hirai Y, Choi YL, Kaneda R, Fujiwara S-i, Arai Y, Akutsu M, Tsutsumi C, Miyazaki Y, Usuki K, Teramura M, Mitani K, Kano Y, O'Neill MC, Urabe A, Tomonaga M, Ozawa K & Mano H: Gene expression profiles of CD133-positive fractions predict the survival of individuals with acute myeloid leukemia. *Cancer Genomics & Proteomics*, **3:** 169-182, 2006.

5) Yamada T, Katagiri H, Ishigaki Y, Ogihara T, Imai J, Uno K, Hasegawa Y, Gao J, Ishihara H, Niijima A, Mano H, Aburatani H, Asano T & Oka Y: Signals from intra-abdominal fat modulate insulin and leptin sensitivity through different mechanisms: neuronal involvement in food-intake regulation. *Cell Metab*, **3:** 223-229, 2006.

6) Takada S, Wada T, Kaneda R, Choi YL, Yamashita Y & Mano H: Evidence for activation of Amh gene expression by steroidogenic factor 1. *Mech Dev*, **123:** 472-480, 2006.

7) Takada S, Ota J, Kansaku N, Yamashita H, Izumi T, Ishikawa M, Wada T, Kaneda R, Choi YL, Koinuma K, Fujiwara S, Aoki H, Kisanuki H, Yamashita Y & Mano H: Nucleotide sequence and embryonic expression of quail and duck Sox9 genes. *Gen Comp Endocrinol*, **145:** 208-213, 2006.

8) Takada S, Berezikov E, Yamashita Y, Lagos-Quintana M, Kloosterman WP, Enomoto M, Hatanaka H, Fujiwara S, Watanabe H, Soda M, Choi YL, Plasterk

RH, Cuppen E & Mano H: Mouse microRNA profiles determined with a new and sensitive cloning method. *Nucleic Acids Res*, **34**: e115, 2006.
9) Taguchi J, Miyazaki Y, Tsutsumi C, Sawayama Y, Ando K, Tsushima H, Fukushima T, Hata T, Yoshida S, Kuriyama K, Honda S, Jinnai I, Mano H & Tomonaga M: Expression of the myeloperoxidase gene in AC133 positive leukemia cells relates to the prognosis of acute myeloid leukemia. *Leuk Res*, **30**: 1105-1112, 2006.
10) Omi T, Kumada M, Kamesaki T, Okuda H, Munkhtulga L, Yanagisawa Y, Utsumi N, Gotoh T, Hata A, Soma M, Umemura S, Ogihara T, Takahashi N, Tabara Y, Shimada K, Mano H, Kajii E, Miki T & Iwamoto S: An intronic variable number of tandem repeat polymorphisms of the cold-induced autoinflammatory syndrome 1 (CIAS1) gene modifies gene expression and is associated with essential hypertension. *Eur J Hum Genet*, 2006.
11) Mano H: Epigenetics and hematological disorders. *Rinsho Ketsueki*, **47**: 3-8, 2006.
12) Mano H: DNA microarray analysis of myelodysplastic syndrome. *Leuk Lymphoma*, **47**: 9-14, 2006.
13) Koinuma K, Yamashita Y, Liu W, Hatanaka H, Kurashina K, Wada T, Takada S, Kaneda R, Choi YL, Fujiwara SI, Miyakura Y, Nagai H & Mano H: Epigenetic silencing of AXIN2 in colorectal carcinoma with microsatellite instability. *Oncogene*, **25**: 139-146, 2006.
14) Kano Y, Akutsu M, Tsunoda S, Izumi T, Kobayashi H, Inoue K, Mori K, Fujii H, Mano H, Odgerel T & Furukawa Y: Schedule-dependent interactions between pemetrexed and cisplatin in human carcinoma cell lines in vitro. *Oncol Res*, **16**: 85-95, 2006.
15) Choi YL, Tsukasaki K, O'Neill M C, Yamada Y, Onimaru Y, Matsumoto K, Ohashi J, Yamashita Y, Tsutsumi S, Kaneda R, Takada S, Aburatani H, Kamihira S, Nakamura T, Tomonaga M & Mano H: A genomic analysis of adult T-cell leukemia. *Oncogene*, 2006.
16) Berezikov E, van Tetering G, Verheul M, van de Belt J, van Laake L, Vos J, Verloop R, van de Wetering M, Guryev V, Takada S, van Zonneveld AJ, Mano H, Plasterk R & Cuppen E: Many novel mammalian microRNA candidates identified by extensive cloning and RAKE analysis. *Genome Res*, **16**: 1289-1298, 2006.
17) Takada S, Mano H & Koopman P: Regulation of Amh during sex determination in chickens: Sox gene expression in male and female gonads. *Cell Mol Life Sci*, **62**: 2140-2146, 2005.
18) Ohki R, Yamamoto K, Ueno S, Mano H, Misawa Y, Fuse K, Ikeda U & Shimada K: Gene expression profiling of human atrial myocardium with atrial fibrillation by DNA microarray analysis. *Int J Cardiol*, **102**: 233-238, 2005.
19) Numata A, Shimoda K, Kamezaki K, Haro T, Kakumitsu H, Shide K, Kato K, Miyamoto T, Yamashita Y, Oshima Y, Nakajima H, Iwama A, Aoki K, Takase K, Gondo H, Mano H & Harada M: Signal transducers and activators of transcription 3 augments the transcriptional activity of CCAAT/enhancer-binding protein alpha in granulocyte colony-stimulating factor signaling pathway. *J Biol Chem*, **280**: 12621-12629, 2005.
20) Koinuma K, Kaneda R, Toyota M, Yamashita Y, Takada S, Choi YL, Wada T, Okada M, Konishi F, Nagai H & Mano H: Screening for genomic fragments that are methylated specifically in colorectal carcinoma with a methylated MLH1 promoter. *Carcinogenesis*, **26**: 2078-2085, 2005.
21) Kisanuki H, Choi YL, Wada T, Moriuchi R, Fujiwara SI, Kaneda R, Koinuma K, Ishikawa M, Takada S, Yamashita Y & Mano H: Retroviral expression screening of oncogenes in pancreatic ductal

carcinoma. *Eur J Cancer*, **41:** 2170-2175, 2005.
22) Kaneda R, Ueno S, Yamashita Y, Choi YL, Koinuma K, Takada S, Wada T, Shimada K & Mano H: Genome-wide screening for target regions of histone deacetylases in cardiomyocytes. *Circ Res*, **97:** 210-218, 2005.
23) Ishikawa M, Yoshida K, Yamashita Y, Ota J, Takada S, Kisanuki H, Koinuma K, Choi YL, Kaneda R, Iwao T, Tamada K, Sugano K & Mano H: Experimental trial for diagnosis of pancreatic ductal carcinoma based on gene expression profiles of pancreatic ductal cells. *Cancer Sci*, **96:** 387-393, 2005.
24) Fujiwara S, Yamashita Y, Choi YL, Wada T, Kaneda R, Takada S, Maruyama Y, Ozawa K & Mano H: Transforming activity of the lymphotoxin-beta receptor revealed by expression screening. *Biochem Biophys Res Commun*, **338:** 1256-1262, 2005.
25) Choi YL, Moriuchi R, Osawa M, Iwama A, Makishima H, Wada T, Kisanuki H, Kaneda R, Ota J, Koinuma K, Ishikawa M, Takada S, Yamashita Y, Oshimi K & Mano H: Retroviral expression screening of oncogenes in natural killer cell leukemia. *Leuk Res*, **29:** 943-949, 2005.
26) Tsutsumi C, Ueda M, Miyazaki Y, Yamashita Y, Choi YL, Ota J, Kaneda R, Koinuma K, Fujiwara S, Kisanuki H, Ishikawa M, Ozawa K, Tomonaga M & Mano H: DNA microarray analysis of dysplastic morphology associated with acute myeloid leukemia. *Exp Hematol*, **32:** 828-835, 2004.
27) Ohki-Kaneda R, Ohashi J, Yamamoto K, Ueno S, Ota J, Choi YL, Koinuma K, Yamashita Y, Misawa Y, Fuse K, Ikeda U, Shimada K & Mano H: Cardiac function-related gene expression profiles in human atrial myocytes. *Biochem Biophys Res Commun*, **320:** 1328-1336, 2004.
28) Ohki R, Yamamoto K, Ueno S, Mano H, Misawa Y, Fuse K, Ikeda U & Shimada K: Transcriptional profile of genes induced in human atrial myocardium with pressure overload. *Int J Cardiol*, **96:** 381-387, 2004.
29) Mano H: Stratification of acute myeloid leukemia based on gene expression profiles. *Int J Hematol*, **80:** 389-394, 2004.
30) Koinuma K, Shitoh K, Miyakura Y, Furukawa T, Yamashita Y, Ota J, Ohki R, Choi YL, Wada T, Konishi F, Nagai H & Mano H: Mutations of BRAF are associated with extensive hMLH1 promoter methylation in sporadic colorectal carcinomas. *Int J Cancer*, **108:** 237-242, 2004.
31) Kano Y, Akutsu M, Tsunoda S, Izumi T, Mori K, Fujii H, Yazawa Y, Mano H & Furukawa Y: Schedule-dependent synergism and antagonism between pemetrexed and paclitaxel in human carcinoma cell lines in vitro. *Cancer Chemother Pharmacol*, **54:** 505-513, 2004.
32) Kaneda R, Toyota M, Yamashita Y, Koinuma K, Choi YL, Ota J, Kisanuki H, Ishikawa M, Takada S, Shimada K & Mano H: High-throughput screening of genome fragments bound to differentially acetylated histones. *Genes Cells*, **9:** 1167-1174, 2004.
33) He H, Hirokawa Y, Gazit A, Yamashita Y, Mano H, Kawakami Y, Kawakami, Hsieh CY, Kung HJ, Lessene G, Baell J, Levitzki A & Maruta H: The Tyr-kinase inhibitor AG879, that blocks the ETK-PAK1 interaction, suppresses the RAS-induced PAK1 activation and malignant transformation. *Cancer Biol Ther*, **3:** 96-101, 2004.
34) Choi YL, Makishima H, Ohashi J, Yamashita Y, Ohki R, Koinuma K, Ota J, Isobe Y, Ishida F, Oshimi K & Mano H: DNA microarray analysis of natural killer cell-type lymphoproliferative disease of granular lymphocytes with purified CD3(-)CD56(+) fractions. *Leukemia*, **18:** 556-565, 2004.
35) Bai J, Sata N, Nagai H, Wada T, Yoshida K, Mano H, Sata F & Kishi R: Genistein-Induced Changes in Gene

Expression in Panc 1 Cells at Physiological Concentrations of Genistein. *Pancreas*, **29:** 93-98, 2004.
36) Araki H, Katayama N, Yamashita Y, Mano H, Fujieda A, Usui E, Mitani H, Ohishi K, Nishii K, Masuya M, Minami N, Nobori T & Shiku H: Reprogramming of human postmitotic neutrophils into macrophages by growth factors. *Blood*, **103:** 2973-2980, 2004.
37) Aoki N, Ueno S-i, Mano H, Yamasaki S, Shiota M, Miyazaki H, Yamaguchi-Aoki Y, Matsuda T & Ullrich A: Mutual regulation of protein-tyrosine phosphatase 20 and protein-tyrosine kinase Tec activities by tyrosine phosphorylation and dephosphorylation. J Biol Chem, 279: 10765-10775, 2004.

寺村正尚

1) Okamoto H, Teramura M & Kamatani N: Myelodysplastic syndrome associated with low-dose methotrexate in rheumatoid arthritis. *Ann Pharmacother*, **38:** 172-173, 2004.
2) Fujimura K, Kuwana M, Kurata Y, Imamura M, Harada H, Sakamaki H, Teramura M, Koda K, Nomura S, Sugihara S, Shimomura T, Fujimoto TT, Oyashiki K & Ikeda Y: Is eradication therapy useful as the first line of treatment in Helicobacter pylori-positive idiopathic thrombocytopenic purpura? Analysis of 207 eradicated chronic ITP cases in Japan. *Int J Hematol*, **81:** 162-168, 2005.
3) Tsuchiya K, Saito M, Okano-Sugiyama H, Nihei H, Ando M, Teramura M, Iwamoto YS, Shimada K & Akiba T: Monitoring the content of reticulocyte hemoglobin (CHr) as the progression of anemia in nondialysis chronic renal failure (CRF) patients. *Ren Fail*, **27:** 59-65, 2005.
4) Araki T, Emoto M, Teramura M, Yokoyama H, Mori K, Hatsuda S, Maeno T, Shinohara K, Koyama H, Shoji T, Inaba M & Nishizawa Y: Effect of adiponectin on carotid arterial stiffness in type 2 diabetic patients treated with pioglitazone and metformin. *Metabolism*, **55:** 996-1001, 2006.
5) Ishiyama M, Teramura M, Iwabe K, Kato T & Motoji T: Clonally expanded T-cells in the peripheral blood of patients with idiopathic Thrombocytopenic purpura and Helicobacter pylori infection. *Int J Hematol*, **83:** 147-151, 2006.
6) Mori K, Emoto M, Yokoyama H, Araki T, Teramura M, Koyama H, Shoji T, Inaba M & Nishizawa Y: Association of serum fetuin-A with insulin resistance in type 2 diabetic and nondiabetic subjects. *Diabetes Care*, **29:** 468, 2006.
7) Sugimori C, Chuhjo T, Feng X, Yamazaki H, Takami A, Teramura M, Mizoguchi H, Omine M & Nakao S: Minor population of CD55-CD59- blood cells predicts response to immunosuppressive therapy and prognosis in patients with aplastic anemia. *Blood*, **107:** 1308-1314, 2006.
8) Teramura M: [Anemia due to hematopoietic disorders. 1. Aplastic anemia and pre red cell aplasia]. *Nippon Naika Gakkai Zasshi*, **95:** 2030-2035, 2006.
9) Yokoyama H, Emoto M, Mori K, Araki T, Teramura M, Koyama H, Shoji T, Inaba M & Nishizawa Y: Plasma adiponectin level is associated with insulin-stimulated nonoxidative glucose disposal. *J Clin Endocrinol Metab*, **91:** 290-294, 2006.

湯尾明

1) Nakai-Murakami C, Shimura M, Kinomoto M, Takizawa Y, Tokunaga K, Taguchi T, Hoshino S, Miyagawa K, Sata T, Kurumizaka H, Yuo A & Ishizaka Y: HIV-1 Vpr induces ATM-dependent cellular signal with enhanced homologous recombination. *Oncogene*, **26:** 477-486, 2007.
2) Zhang H, Saeki K, Kimura A, Saeki K, Nakahara M, Doshi M, Kondo Y, Nakano T & Yuo A: Efficient and repetitive production of hematopoietic and endothelial cells from feeder-free

monolayer culture system of primate embryonic stem cells. *Biol Reprod*, **74**: 295-306, 2006.
3) Yasugi E, Horiuchi A, Uemura I, Okuma E, Nakatsu M, Saeki K, Kamisaka Y, Kagechika H, Yasuda K & Yuo A: Peroxisome proliferator-activated receptor gamma ligands stimulate myeloid differentiation and lipogenensis in human leukemia NB4 cells. *Dev Growth Differ*, **48**: 177-188, 2006.
4) Doshi M, Koyanagi M, Nakahara M, Saeki K, Saeki K & Yuo A: Identification of human neutrophils during experimentally induced inflammation in mice with transplanted CD34+ cells from human umbilical cord blood. *Int J Hematol*, **84**: 231-237, 2006.
5) Saeki K, Yasugi E, Okuma E, Breit SN, Nakamura M, Toda T, Kaburagi Y & Yuo A: Proteomic analysis on insulin signaling in human hematopoietic cells: identification of CLIC1 and SRp20 as novel downstream effectors of insulin. *Am J Physiol Endocrinol Metab*, **289**: E419-428, 2005.
6) Nakatsu M, Doshi M, Saeki K & Yuo A: Synergistic effects of dehydroepiandrosterone and retinoic acid on granulocytic differentiation of human promyelocytic NB4 cells. *Int J Hematol*, **81**: 32-38, 2005.

H. 知的財産権の出願・登録状況

国際公開番号：PCT/WO97/34007・発明者：間野博行・名称「PROMOTER」・出願人：間野博行、株式会社 DNAVEC 研究所・公開日：1997 年 9 月 18 日

公開番号：特開 2001-269174・発明者：間野博行・名称「骨髄異形成症候群(MDS)の検出方法及び MDS の治療剤」・出願人：間野博行・公開日：2001 年 10 月 2 日

国際公開番号：PCT/WO 01/64946 A1・発明者:間野博行・名称「METHOD OF DETECTING CHRONIC MYELOGENOUS LEUKEMIA」・出願人：間野博行、宝酒造株式会社・公開日：2001 年 9 月 7 日

出願番号：特願 2001-337752・発明者：間野博行・名称「多発性骨髄腫の分子診断方法」・出願人：藤沢薬品工業工業株式会社・出願日：2001 年 11 月 2 日

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出願番号：特願 2004-505392・発明者：間野博行・名称「膵管細胞を利用した膵管癌特異的遺伝子の同定方法、同方法により同定される膵管癌特異的遺伝子を利用した膵管癌の検査方法、および膵管癌の治療または予防のための医薬候補化合物のスクリーニング方法」・出願人：藤沢薬品工業株式会社・出願日 2003 年 5 月 22 日・国際出願番号：PCT/JP/03/006398

出願番号：特願２００５－１６８３３６。出願日：平成１７年６月８日。発明名称：成人T細胞白血病予防治療剤

米国国際出願番号：10/514235。発明名称：Method of identifying pancreatic ductal carcinoma-specific genes using pancreatic ductal cells
カナダ国際出願番号：2486028。発明名称：Method of identifying pancreatic ductal carcinoma-specific genes using pancreatic ductal cells

出願番号：特願２００６－３０３９２９。「霊長類動物胚性幹細胞の培養及び継代方法、並びにその分化誘導方法」発明者：湯尾明、佐伯久美子、佐伯晃一、中原正子、中村直子、過足芳子、松山さと子、米田麻子。出願人：国立国際医療センター、田辺製薬株式会社

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発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Yamashita Y, Minoura K, Taya T, Fujiwara S-i, Kurashina K, Watanabe H, Choi YL, Soda M, Hatanaka H, Enomoto M, Takada S & Mano H	Analysis of chromosome copy number in leukemic cells by different microarray platforms.	*Leukemia*	***in press***		2007
Kano Y, Akutsu M, Tsunoda S, Izumi T, Kobayashi H, Mano H & Furukawa Y	Cytotoxic effects of histone deacetylase inhibitor FK228 (depsipeptide, formally named FR901228) in combination with conventional anti-leukemia/lymphoma agents against human leukemia/lymphoma cell lines.	*Invest New Drugs*	**25**	31-40	2007
Choi YL, Kaneda R, Wada T, Fujiwara S, Soda M, Watanabe H, Kurashina K, Hatanaka H, Enomoto M, Takada S, Yamashita Y & Mano H	Identification of a constitutively active mutant of JAK3 by retroviral expression screening.	*Leuk Res*	**31**	203-209	2007
Yamashita Y, Ohashi J, Hirai Y, Choi YL, Kaneda R, Fujiwara S-i, Arai Y, Akutsu M, Tsutsumi C, Miyazaki Y, Usuki K, Teramura M, Mitani K, Kano Y, O'Neill MC, Urabe A, Tomonaga M, Ozawa K & Mano H	Gene expression profiles of CD133-positive fractions predict the survival of individuals with acute myeloid leukemia.	*Cancer Genomics & Proteomics,*	**3**	169-182	2006
Yamada T, Katagiri H, Ishigaki Y, Ogihara T, Imai J, Uno K, Hasegawa Y, Gao J, Ishihara H, Niijima A, Mano H, Aburatani H, Asano T & Oka Y	Signals from intra-abdominal fat modulate insulin and leptin sensitivity through different mechanisms: neuronal involvement in food-intake regulation.	*Cell Metab*	**3**	223-229	2006
Takada S, Wada T, Kaneda R, Choi YL, Yamashita Y & Mano H	Evidence for activation of Amh gene expression by steroidogenic factor 1.	*Mech Dev*	**123**	472-480	2006
Takada S, Ota J, Kansaku N, Yamashita H, Izumi T, Ishikawa M, Wada T, Kaneda R, Choi YL, Koinuma K, Fujiwara S, Aoki H, Kisanuki H, Yamashita Y & Mano H	Nucleotide sequence and embryonic expression of quail and duck Sox9 genes.	*Gen Comp Endocrinol*	**145**	208-213	2006
Takada S, Berezikov E, Yamashita Y, Lagos-Quintana M, Kloosterman WP, Enomoto M, Hatanaka H, Fujiwara S, Watanabe H, Soda M, Choi YL, Plasterk RH, Cuppen E & Mano H	Mouse microRNA profiles determined with a new and sensitive cloning method.	*Nucleic Acids Res*	**34**	e115	2006
Taguchi J, Miyazaki Y, Tsutsumi C, Sawayama Y, Ando K, Tsushima H, Fukushima T, Hata T, Yoshida S, Kuriyama K, Honda S, Jinnai I, Mano H & Tomonaga M	Expression of the myeloperoxidase gene in AC133 positive leukemia cells relates to the prognosis of acute myeloid leukemia	*Leuk Res,*	**30**	1105-1112	2006
Omi T, Kumada M, Kamesaki T, Okuda H, Munkhtulga L, Yanagisawa Y, Utsumi N, Gotoh T, Hata A, Soma M, Umemura S, Ogihara T, Takahashi N, Tabara Y, Shimada K, Mano H, Kajii E, Miki T & Iwamoto S	An intronic variable number of tandem repeat polymorphisms of the cold-induced autoinflammatory syndrome 1 (CIAS1) gene modifies gene expression and is associated with essential hypertension	*Eur J Hum Gene*			2006
Mano H	[Epigenetics and hematological disorders].	*Rinsho Ketsueki*	**47**	3－8	2006
Mano H	DNA microarray analysis of myelodysplastic syndrome	*Leuk Lymphoma*	**47**	9—14	2006
Koinuma K, Yamashita Y, Liu W, Hatanaka H, Kurashina K, Wada T, Takada S, Kaneda R, Choi YL, Fujiwara SI, Miyakura Y, Nagai H & Mano H	Epigenetic silencing of AXIN2 in colorectal carcinoma with microsatellite instability.	*Oncogene*	**25**	139-146	2006

Kano Y, Akutsu M, Tsunoda S, Izumi T, Kobayashi H, Inoue K, Mori K, Fujii H, Mano H, Odgerel T & Furukawa Y	Schedule-dependent interactions between pemetrexed and cisplatin in human carcinoma cell lines in vitro.	*Oncol Res*	16	85-95	2006
Choi YL, Tsukasaki K, O'Neill M C, Yamada Y, Onimaru Y, Matsumoto K, Ohashi J, Yamashita Y, Tsutsumi S, Kaneda R, Takada S, Aburatani H, Kamihira S, Nakamura T, Tomonaga M & Mano H	A genomic analysis of adult T-cell leukemia.	*Oncogene*			2006
Berezikov E, van Tetering G, Verheul M, van de Belt J, van Laake L, Vos J, Verloop R, van de Wetering M, Guryev V, Takada S, van Zonneveld AJ, Mano H, Plasterk R & Cuppen E	Many novel mammalian microRNA candidates identified by extensive cloning and RAKE analysis.	*Genome Res*	16	1289-1298	2006
Takada, S., Mano, H. & Koopman, P.	Regulation of Amh during sex determination in chickens: Sox gene expression in male and female gonads	*Cell. Mol. Life Sci.*	62	2140-2146	2005
Ohki, R., Yamamoto, K., Ueno, S., Mano, H., Misawa, Y., Fuse, K., Ikeda, U. & Shimada, K.	Gene expression profiling of human atrial myocardium with atrial fibrillation by DNA microarray analysis	*Int. J. Cardiol.*	102	233-238	2005
Numata, A., Shimoda, K., Kamezaki, K., Haro, T., Kakumitsu, H., Shide, K., Kato, K., Miyamoto, T., Yamashita, Y., Oshima, Y., Nakajima, H., Iwama, A., Aoki, K., Takase, K., Gondo, H., Mano, H. & Harada, M.	Signal transducers and activators of transcription 3 augments the transcriptional activity of CCAAT/enhancer-binding protein alpha in granulocyte colony-stimulating factor signaling pathway	*J. Biol. Chem.*	280	12621-12629	2005
Koinuma, K., Kaneda, R., Toyota, M., Yamashita, Y., Takada, S., Choi, Y.L., Wada, T., Okada, M., Konishi, F., Nagai, H. & Mano, H.	Screening for genomic fragments that are methylated specifically in colorectal carcinoma with a methylated MLH1 promoter	*Carcinogenesis*	26	2078-2085	2005
Kisanuki, H., Choi, Y.L., Wada, T., Moriuchi, R., Fujiwara, S.I., Kaneda, R., Koinuma, K., Ishikawa, M., Takada, S., Yamashita, Y. & Mano, H.	Retroviral expression screening of oncogenes in pancreatic ductal carcinoma	*Eur. J. Cancer*	41	2170-2175	2005
Kaneda, R., Ueno, S., Yamashita, Y., Choi, Y.L., Koinuma, K., Takada, S., Wada, T., Shimada, K. & Mano, H.	Genome-wide screening for target regions of histone deacetylases in cardiomyocytes	*Circ. Res.*,	97	210-218	2005
Ishikawa, M., Yoshida, K., Yamashita, Y., Ota, J., Takada, S., Kisanuki, H., Koinuma, K., Choi, Y.L., Kaneda, R., Iwao, T., Tamada, K., Sugano, K. & Mano, H.	Experimental trial for diagnosis of pancreatic ductal carcinoma based on gene expression profiles of pancreatic ductal cells	*Cancer Sci*	96	387-393	2005
Fujiwara, S., Yamashita, Y., Choi, Y.L., Wada, T., Kaneda, R., Takada, S., Maruyama, Y., Ozawa, K. & Mano, H.	Transforming activity of the lymphotoxin-beta receptor revealed by expression screening	*Biochem. Biophys. Res. Commun*	338	1256-1262	2005
Choi, Y.L., Moriuchi, R., Osawa, M., Iwama, A., Makishima, H., Wada, T., Kisanuki, H., Kaneda, R., Ota, J., Koinuma, K., Ishikawa, M., Takada, S., Yamashita, Y., Oshimi, K. & Mano, H	Retroviral expression screening of oncogenes in natural killer cell leukemia	*Leuk. Res*	29	943-949	2005
Tsutsumi, C., Ueda, M., Miyazaki, Y., Yamashita, Y., Choi, Y.L., Ota, J., Kaneda, R., Koinuma, K., Fujiwara, S., Kisanuki, H., Ishikawa, M., Ozawa, K., Tomonaga, M. & Mano, H.	DNA microarray analysis of dysplastic morphology associated with acute myeloid leukemia	*Exp. Hematol*	32	828-835	2004
Ohki-Kaneda, R., Ohashi, J., Yamamoto, K., Ueno, S., Ota, J., Choi, Y.L., Koinuma, K., Yamashita, Y., Misawa, Y., Fuse, K., Ikeda, U., Shimada, K. & Mano, H.	Cardiac function-related gene expression profiles in human atrial myocytes	*Biochem. Biophys. Res. Commun.*	320	1328-1336	2004
Ohki, R., Yamamoto, K., Ueno, S., Mano, H., Misawa, Y., Fuse, K., Ikeda, U. & Shimada, K.	Transcriptional profile of genes induced in human atrial myocardium with pressure overload	*Int. J. Cardiol*	96	381-387	2004
Mano, H.	Stratification of Acute Myeloid Leukemia Based on Gene Expresstion Profiles	*Int.J.Hematol.*	80	389-394	2004

Koinuma, K., Shitoh, K., Miyakura, Y., Furukawa, T., Yamashita, Y., Ota, J., Ohki, R., Choi, Y.L., Wada, T., Konishi, F., Nagai, H. & Mano, H.	Mutations of BRAF are associated with extensive hMLH1 promoter methylation in sporadic colorectal carcinomas	*Int. J. Cancer*	108	237-242	2004
Kano, Y., Akutsu, M., Tsunoda, S., Izumi, T., Mori, K., Fujii, H., Yazawa, Y., Mano, H. & Furukawa, Y.	Schedule-dependent synergism and antagonism between pemetrexed and paclitaxel in human carcinoma cell lines in vitro	*Cancer Chemother. Pharmacol*	54	505-513	2004
Kaneda, R., Toyota, M., Yamashita, Y., Koinuma, K., Choi, Y.L., Ota, J., Kisanuki, H., Ishikawa, M., Takada, S., Shimada, K. & Mano, H.	High-throughput screening of genome fragments bound to differentially acetylated histones	*Genes Cells*	9	1167-1174	2004
He, H., Hirokawa, Y., Gazit, A., Yamashita, Y., Mano, H., Kawakami, Y., Kawakami, Hsieh, C.Y., Kung, H.J., Lessene, G., Baell, J., Levitzki, A. & Maruta, H.	The Tyr-Kinase Inhibitor AG879, That Blocks the ETK-PAK1 Interaction, Suppresses the RAS-Induced PAK1 Activation and Malignant Transformation	*Cancer Biol. Ther.*	3	96-101	2004
Choi, Y.L., Makishima, H., Ohashi, J., Yamashita, Y., Ohki, R., Koinuma, K., Ota, J., Isobe, Y., Ishida, F., Oshimi, K. & Mano, H.	DNA microarray analysis of natural killer cell-type lymphoproliferative disease of granular lymphocytes with purified CD3(-)CD56(+) fractions	*Leukemia*	18	556-565	2004
Bai J, Sata N, Nagai H, Wada T, Yoshida K, Mano H, Sata F & Kishi R	Genistein-Induced Changes in Gene Expression in Panc 1 Cells at Physiological Concentrations of Genistein.	*Pancreas*	29	93-98	2004
Araki H, Katayama N, Yamashita Y, Mano H, Fujieda A, Usui E, Mitani H, Ohishi K, Nishii K, Masuya M, Minami N, Nobori T & Shiku H	Reprogramming of human postmitotic neutrophils into macrophages by growth factors.	*Blood*	103	2973-2980	2004
Aoki N, Ueno S-i, Mano H, Yamasaki S, Shiota M, Miyazaki H, Yamaguchi-Aoki Y, Matsuda T & Ullrich A	Mutual regulation of protein-tyrosine phosphatase 20 and protein-tyrosine kinase Tec activities by tyrosine phosphorylation and dephosphorylation.	*J Biol Chem*	279	10765-10775	2004

研究成果の刊行に関する一覧表

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発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Araki T, Emoto M, Teramura M, Yokoyama H, Mori K, Hatsuda S, Maeno T, Shinohara K, Koyama H, Shoji T, Inaba M & Nishizawa Y	Effect of adiponectin on carotid arterial stiffness in type 2 diabetic patients treated with pioglitazone and metformin	*Metabolism*	55	996-1001	2006
Ishiyama M, Teramura M, Iwabe K, Kato T & Motoji T	Clonally expanded T-cells in the peripheral blood of patients with idiopathic Thrombocytopenic purpura and Helicobacter pylori infection.	*Int J Hematol*	83	147-151	2006
Mori K, Emoto M, Yokoyama H, Araki T, Teramura M, Koyama H, Shoji T, Inaba M & Nishizawa Y	Association of serum fetuin-A with insulin resistance in type 2 diabetic and nondiabetic subjects	*Diabetes Care*	29	468	2006
Sugimori C, Chuhjo T, Feng X, Yamazaki H, Takami A, Teramura M, Mizoguchi H, Omine M & Nakao S	Minor population of CD55-CD59- blood cells predicts response to immunosuppressive therapy and prognosis in patients with aplastic anemia	*Blood*	107	1308-1314	2006
Teramura M	[Anemia due to hematopoietic disorders. 1. Aplastic anemia and pre red cell aplasia].	*Nippon Naika Gakkai Zasshi*	95	2030-2035	2006
Yokoyama H, Emoto M, Mori K, Araki T, Teramura M, Koyama H, Shoji T, Inaba M & Nishizawa Y	Plasma adiponectin level is associated with insulin-stimulated nonoxidative glucose disposal	*J Clin Endocrinol Metab*	91	290-294	2006
Fujimura K, Kuwana M, Kurata Y, Imamura M, Harada H, Sakamaki H, Teramura M, Koda K, Nomura S, Sugihara S, Shimomura T, Fujimoto TT, Oyashiki K & Ikeda Y	Is eradication therapy useful as the first line of treatment in Helicobacter pylori-positive idiopathic thrombocytopenic purpura? Analysis of 207 eradicated chronic ITP cases in Japan.	*Int J Hematol*	81	162-168	2005
Tsuchiya K, Saito M, Okano-Sugiyama H, Nihei H, Ando M, Teramura M, Iwamoto YS, Shimada K & Akiba T	Monitoring the content of reticulocyte hemoglobin (CHr) as the progression of anemia in nondialysis chronic renal failure (CRF) patients	*Ren Fail*	27	59-65	2005
Okamoto H, Teramura M & Kamatani N	Myelodysplastic syndrome associated with low-dose methotrexate in rheumatoid arthritis.	*Ann Pharmacother*	38	172-173	2005

研究成果の刊行に関する一覧表

分担研究者:湯尾 明

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Nakai-Murakami C, Shimura M, Kinomoto M, Takizawa Y, Tokunaga K, Taguchi T, Hoshino S, Miyagawa K, Sata T, Kurumizaka H, Yuo A & Ishizaka Y	HIV-1 Vpr induces ATM-dependent cellular signal with enhanced homologous recombination.	*Oncogene*	**26**	477-486	2007
Zhang H, Saeki K, Kimura A, Saeki K, Nakahara M, Doshi M, Kondo Y, Nakano T & Yuo A	Efficient and repetitive production of hematopoietic and endothelial cells from feeder-free monolayer culture system of primate embryonic stem cells.	*Biol Reprod*	**74**	295-306	2006
Yasugi E, Horiuchi A, Uemura I, Okuma E, Nakatsu M, Saeki K, Kamisaka Y, Kagechika H, Yasuda K & Yuo A	Peroxisome proliferator-activated receptor gamma ligands stimulate myeloid differentiation and lipogenensis in human leukemia NB4 cells.	*Dev Growth Differ*	**48**	177-188	2006
Doshi M, Koyanagi M, Nakahara M, Saeki K, Saeki K & Yuo A	Identification of human neutrophils during experimentally induced inflammation in mice with transplanted CD34+ cells from human umbilical cord blood.	*Int J Hematol*	**84**	231-237	2006
Saeki K, Yasugi E, Okuma E, Breit SN, Nakamura M, Toda T, Kaburagi Y & Yuo A	Proteomic analysis on insulin signaling in human hematopoietic cells: identification of CLIC1 and SRp20 as novel downstream effectors of insulin.	*Am J Physiol Endocrinol Metab*	**289**	E419-E428	2005
Nakatsu M, Doshi M, Saeki K & Yuo A	Synergistic effects of dehydroepiandrosterone and retinoic acid on granulocytic differentiation of human promyelocytic NB4 cells	*Int J Hematol*	**81**	32-38	2005

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ONCOGENOMICS

A genomic analysis of adult T-cell leukemia

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Adult T-cell leukemia (ATL) is an intractable malignancy of CD4$^+$ T cells that is etiologically associated with infection by human T-cell leukemia virus-type I. Most individuals in the chronic stage of ATL eventually undergo progression to a highly aggressive acute stage. To clarify the mechanism responsible for this stage progression, we isolated CD4$^+$ cells from individuals in the chronic ($n=19$) or acute ($n=22$) stages of ATL and subjected them to profiling of gene expression with DNA microarrays containing >44 000 probe sets. Changes in chromosome copy number were also examined for 24 cell specimens with the use of microarrays harboring ~50 000 probe sets. Stage-dependent changes in gene expression profile and chromosome copy number were apparent. Furthermore, expression of the gene for MET, a receptor tyrosine kinase for hepatocyte growth factor (HGF), was shown to be specific to the acute stage of ATL, and the plasma concentration of HGF was increased in individuals in either the acute or chronic stage. HGF induced proliferation of a MET-positive ATL cell line, and this effect was blocked by antibodies to HGF. The HGF-MET signaling pathway is thus a potential therapeutic target for ATL.
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Introduction

Adult T-cell leukemia (ATL) is an intractable malignancy of CD4$^+$ T cells that is etiologically associated with infection by human T-cell leukemia virus-type I (HTLV-I) (Uchiyama *et al.*, 1977; Poiesz *et al.*, 1980; Yoshida *et al.*, 1982). Virally encoded proteins such as Tax trigger polyclonal growth of T cells in infected individuals, and there are an estimated 15–20 million such carriers worldwide (Edlich *et al.*, 2000). After a latency period of decades, a small proportion of carriers (~2%) develop ATL. Many ATL patients initially manifest only monoclonal (or oligoclonal) growth of leukemic clones without apparent clinical symptoms, a condition referred to as the chronic or smoldering stages (Shimoyama, 1991). Most individuals in the chronic stage, however, eventually undergo progression to a highly aggressive acute stage (Tajima, 1990). Given that the prognosis of individuals at the acute stage remains very poor, it is important to clarify the molecular mechanism that underlies stage progression.

Homozygous deletion or epigenetic silencing of the gene for the cyclin-dependent kinase inhibitor p16 (Hatta *et al.*, 1995; Yamada *et al.*, 1997; Nosaka *et al.*, 2000) as well as altered expression of other genes related to cell proliferation (Cesarman *et al.*, 1992; Tamiya *et al.*, 1998) have been detected in ATL cells at the acute stage. However, such genetic or epigenetic changes may be infrequent (Matsuoka, 2003), and the transforming events responsible for chronic to acute stage progression remain largely unknown.

DNA microarray analysis allows simultaneous comparison of the expression intensities of tens of thousands of genes. Such analysis of the transcriptomes of ATL cells at the chronic and acute stages might thus be expected to provide insight into the mechanism of stage progression in this disease. With the use of this approach, Sasaki *et al.* (2005) recently compared transcriptomes between normal CD4$^+$ T cells ($n=5$) and mononuclear cells (MNCs) isolated from individuals in the acute stage of ATL ($n=8$). Tsukasaki *et al.* (2004) also compared transcriptomes between MNCs from patients in the chronic or acute stages of ATL ($n=4$ for each). However, the significance of these data may be limited by the small number of study subjects and by the use of unfractionated MNCs that contain various proportions of non-ATL cells.

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In addition to changes in gene expression, ATL cells frequently manifest various karyotype anomalies. Comparative genomic hybridization (CGH) has thus revealed recurrent gains in chromosomes 2p, 3p, 7q and 14q as well as losses in 6q in ATL cells (Ariyama *et al.*, 1999; Tsukasaki *et al.*, 2001). However, CGH or its successor, bacterial artificial chromosome (BAC) array-based CGH, is able to analyse chromosome copy number alterations (CNAs) at a resolution of only several hundred kilobase pairs (Lockwood *et al.*, 2005). High-density oligonucleotide microarrays originally designed for genotyping of single nucleotide polymorphisms (SNPs) have recently been adapted for CNA analysis (Lin *et al.*, 2004; Nannya *et al.*, 2005). With this approach, chromosome copy number is inferred from the signal intensity of SNP probe sets distributed throughout the human genome. For instance, with Affymetrix GeneChip Mapping 100K arrays developed for genotyping of ~100 000 SNPs, it is possible to determine CNAs at a mean resolution of 23.6 kbp, which is substantially greater than that achievable with BAC array-based technologies.

With both microarray-based gene expression profiling and SNP array-based CNA profiling, we have now performed a comprehensive genomic analysis of ATL in order to investigate the mechanism of stage progression from chronic to acute. Given that the $CD4^+CD8^-$ fraction of peripheral blood (PB) cells of individuals with chronic or acute ATL is composed predominantly of ATL cells, we purified this fraction from ATL patients. We then subjected the isolated cells to gene expression profiling with microarrays containing >44 000 probe sets and to CNA analysis with microarrays harboring ~50 000 probe sets. The gene expression data indicate that the transcriptomes for the chronic and acute stages of ATL are distinct, and the CNA data reveal frequent amplification or deletion of genomic fragments of various sizes in each ATL stage.

Results

Transcriptomes of ATL cells

To characterize the transcriptomes of ATL cells, we purified $CD4^+$ cells from PB of ATL patients at either the chronic ($n=19$) or the acute ($n=22$) stage. The clinical characteristics of the patients are summarized in Supplementary Table 1. The $CD4^+$ fraction was also purified from healthy volunteers ($n=3$) and was either activated with phytohemagglutinin (PHA) or not.

A simple, one-step column purification with antibodies to CD4 yielded a highly pure $CD3^+CD4^+$ T-cell fraction. For example, whereas the $CD3^+CD4^+$ fraction constituted only 29.1% of PB MNCs of one healthy individual, it constituted 98.8% of the corresponding column eluate (Figure 1a). Similarly, $CD3^+CD4^+$ cells constituted 25.7% of MNCs from one ATL patient at the acute stage, but accounted for 97.5% of cells in the corresponding column eluate (data not shown).

All of the ATL and normal $CD4^+$ cell specimens were then subjected to expression profiling with ~44 000 probe sets (corresponding to ~33 000 transcripts) on Affymetrix HGU133 microarrays. To eliminate from the analysis genes that were transcriptionally silent in the ATL specimens, we first selected probe sets that received the 'Present' call by Microarray Suite 5.0 software (Affymetrix) in at least 30% ($n=13$) of the ATL samples. A total of 15 121 probe sets fulfilled this criterion. On the basis of the similarity of the expression profiles for these probe sets, all 47 samples were subjected to hierarchical two-way clustering (Alon *et al.*, 1999), yielding a dendrogram of the subjects (Figure 1b). All six normal samples, irrespective of PHA stimulation, formed a distinct branch separated from the ATL specimens, indicating that the overall gene expression profiles differed between normal and transformed T cells. However, samples corresponding to patients with chronic or acute ATL were not clearly separated from each other in this tree.

To compare the transcriptomes of ATL cells between chronic and acute stages, we conducted Student's *t*-test on the gene expression intensity for the 15 121 probe sets with the Benjamini and Hochberg false discovery rate (Reiner *et al.*, 2003) of 0.01, leading to the isolation of 84 probe sets (data not shown). To enrich probe sets whose expression level was high in at least one of the stages, we adopted another selection window, effect size (absolute difference in mean expression intensity) (Dhanasekaran *et al.*, 2001). We extensively compared the expression level of given probe sets determined by DNA microarray and by quantitative real-time reverse transcription–polymerase chain reaction (RT–PCR). With our normalization procedure (see Materials and methods), expression of genes with an array data of ≥100 units (U) was almost always detected by real-time RT–PCR (data not shown). Thus, we chose 100 U as the threshold value for the effect size.

A total of 21 probe sets (corresponding to 21 independent genes) whose expression level contrasted the two clinical conditions were finally identified. Hierarchical two-way clustering analysis of the expression profiles of these stage-associated genes revealed that only two gene were preferentially expressed at the chronic stage, whereas the other 19 genes were preferentially expressed in the acute stage (Figure 1c and Supplementary Table 2). Interestingly, the latter gene cluster contains several genes encoding for growth-related proteins, such as nuclear receptor coactivator 3 (NCOA3, GenBank accession no. NM_006534), heat-shock 60-kDa protein 1 (HSPD1, GenBank accession no. NM_002156) and general transcription factor IIIA (GTF3A, GenBank accession no. BE542815).

Gene expression-based prediction of ATL stage

We next attempted to develop a microarray-based class prediction algorithm for ATL. Among several approaches examined, an artificial neural network (ANN) provided the highest accuracy for prediction (O'Neill and Song, 2003). ANNs are computer-based

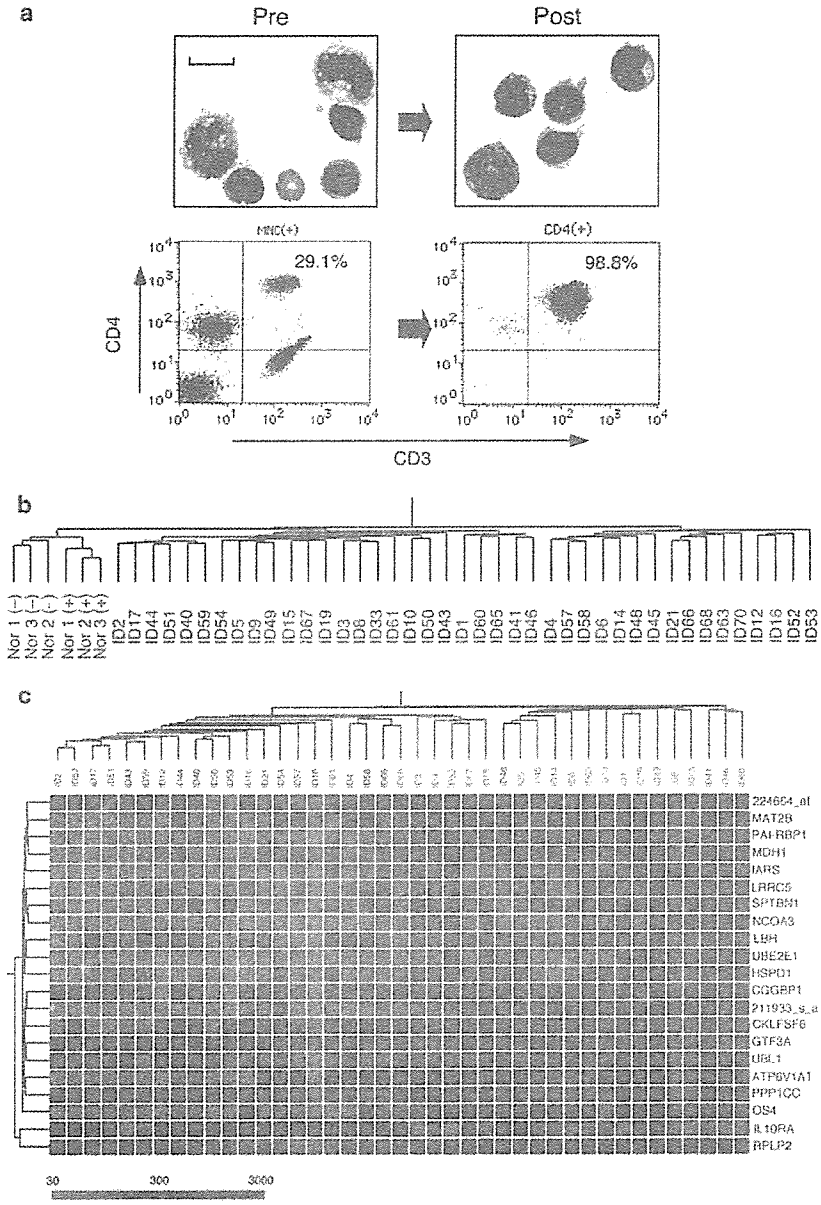

Figure 1 Purification and gene expression profiling of ATL cells. (**a**) MNCs isolated from the PB of a healthy individual were subjected to staining with Wright–Giemsa solution before (Pre) and after (Post) purification by affinity chromatography with antibodies to CD4 (upper panels). Scale bar, 10 μm. The same fractions were also subjected to flow cytometry with antibodies to CD3 and to CD4 (lower panels). The proportion of double-positive cells is indicated. (**b**) Subject tree generated by hierarchical clustering analysis of the expression profiles for 15 121 probe sets. Normal T cells (Nor 1–3) stimulated (+) or not (−) with PHA (8 μg/ml) clustered together, separate from the ATL samples from patients in the chronic (green) or acute (red) stage. (**c**) Subject tree generated by two-way clustering analysis with 21 probe sets that contrasted the two clinical conditions (Student's *t*-test with the Benjamini and Hochberg false discovery rate of 0.01, and effect size of $\geqslant$100 U). Each column corresponds to a separate sample, and each row to a probe set whose expression is color-coded according to the indicated scale. Gene symbols are shown on the right; 224664_at and 211933_s_at are the expressed sequence tag IDs designated by Affymetrix (http://www.affymetrix.com). Annotations and expression intensities for the probe sets are presented in Supplementary Table 2.

algorithms modeled on the structure and behavior of neurons in human brain. Pattern recognition by ANNs is accomplished by training the networks for multiple times in a supervised mode. ANNs adjust continuously their internal weighted connections to reduce the observed errors in matching input to output.

Here, the 15 121 probe sets originally selected in Figure 1b were divided into three nonoverlapping

groups, each of which was used as the input for 10 ANNs (Figure 2a). We performed a 10% crossvalidation rotation with 37 samples, training with 33 samples and testing of the remaining four samples. We then reduced the weight of one input in the first layer (one at a time by 15%), and the network was run again to evaluate the difference in the result from the original output. The same procedure was performed in turn for every input, in order to identify 44 'predictor' genes whose expression markedly influenced the prediction accuracy in each set of ANNs (Figure 2b and Supplementary Table 3). Such predictor set contains only one gene (*UBE2E1*) shared with the stage-associated probe sets shown in Figure 1c. As demonstrated previously, ANN and other approaches (such as *t*-test or clustering analysis) frequently isolate distinct sets of predictor genes (O'Neill and Song, 2003).

Another nine ANNs were then trained and tested with the 44 predictor genes in the same 10% crossvalidation round, yielding one error of prediction for the 37 samples. Finally, the withheld four samples were tested with the trained ANN, resulting in the correct prediction of the class of each. Given that diagnosis of the stage of ATL patients is sometimes problematic, especially when an individual is undergoing stage transition, our analysis offers the possibility of a microarray diagnostic system based on the expression profile of a small number of genes.

Copy number analysis of the ATL genome

To analyse chromosomal gain or loss in ATL cells, we subjected genomic DNA to hybridization with genotyping arrays that represent ~50 000 human SNPs and allow determination of copy number at an average resolution of 47.2 kbp. We first examined whether MNCs and purified $CD4^+$ ATL cells may yield similar CNA profiles by analyzing genomic DNA from such cell fractions of a single individual (patient ID6) at the acute stage of ATL. Flow cytometry revealed that $CD3^+CD4^+$ T cells constituted 58.9 and 98.0% of MNCs and purified $CD4^+$ cells of this individual, respectively (data not shown).

As shown in Figure 3a, gain of chromosomal content ($\geqslant 3n$) was apparent at 1q, 3q, 5p, 7q, 18q and 21q, whereas loss of genomic content ($\leqslant 1n$) was observed at 2p, 12p, 13q, 14q and 18p. In addition to changes affecting such large chromosomal regions, numerous CNAs too small to be detected by conventional methods were apparent at various positions (hospital karyotyping of MNCs from this patient indicated a karyotype of 46,XY). We also identified many chromosomal regions whose copy number differed between the unfractionated MNCs and purified $CD4^+$ cells (Figure 3a). These data indicate that purification of $CD4^+$ cells increases the sensitivity of copy number measurement.

Among the ATL specimens subjected to gene expression profiling, all those for which $CD4^+$ cells were available for preparation of genomic DNA were analysed for CNAs ($n=24$; 15 specimens for the acute stage, nine specimens for the chronic stage). Assessment of copy number revealed frequent anomalies of various sizes, ranging from amplification of an entire chromosome to small deletions spanning only a few probe sets, in the ATL genome (Figure 3b). The most frequent gain or loss in our data set was a small deletion at 14q11.2, which was identified in 22 of the 24 patients tested; the core deleted region spans five probe sets, encompassing as little as 30 857 bp at the locus of *TRD* (encoding T-cell receptor delta locus) and *TRA* (encoding T-cell receptor alpha constant). These deletions likely reflect genomic rearrangement at the T-cell receptor locus in ATL cells and support the high sensitivity of the method.

Further, a high-grade amplification of genome could be found in a region spanning ~14 Mbp at 3p (nucleotide 10 672 576–24 556 563) among the ATL patients, especially at the acute stage. A chromosome copy number of four in this region was inferred for three patients at the acute stage (ID 3, 15 and 70), and that of three was inferred for seven patients. Interestingly, expression level of the genes mapped on this 3p region was significantly higher in the patients with a chromosome copy number of four compared to those with a copy number of two ($P=0.03$, Student's *t*-test), and marginally higher to those with a copy number of three ($P=0.051$) (data not shown).

To confirm the inferred copy numbers in our data set, we subjected genomic DNA at a locus with marked variation in copy number (chromosome 6, nucleotides 16 651 304–16 651 533) to quantitative real-time PCR analysis. Such analysis of the 24 patients, two healthy volunteers (one male, one female), and a cell line (KK-1) (Imaizumi *et al*., 2003) derived from a patient at the acute stage of ATL revealed that the inferred copy number was highly correlated with DNA content measured by PCR (Figure 3c).

Stage-dependent CNAs

To screen for CNA patterns linked to stage progression in ATL, we applied Student's *t*-test ($P<0.01$) to the obtained data set. Subsequent application of a selection window specifying that at least two contiguous probes show the same CNA pattern led to the isolation of 330 probe sets that corresponded to 3p, 3q, 14q and 19p (Figure 3d). Segmental amplification of chromosome 3 was detected only in the ATL patients at the acute stage, consistent with previous results obtained by CGH analysis (Tsukasaki *et al*., 2001; Oshiro *et al*., 2006).

To examine the effect of gene dosage on mRNA abundance, we analysed our gene expression data set for the expression level of genes assigned to a segment (region #1, nucleotides 114 092 369–119 769 881) of chromosome 3 (Figure 3d and e). The mean expression intensity of genes in this region was significantly greater for the patients with a corresponding gain of DNA content than for those without such a gain ($P=0.00015$, Student's *t*-test). Similarly, the expression level of genes on a segment (region #2; nucleotide 8 782 486–12 322 072) of chromosome 19 was greater in cells with a gain of DNA content in this region than in those

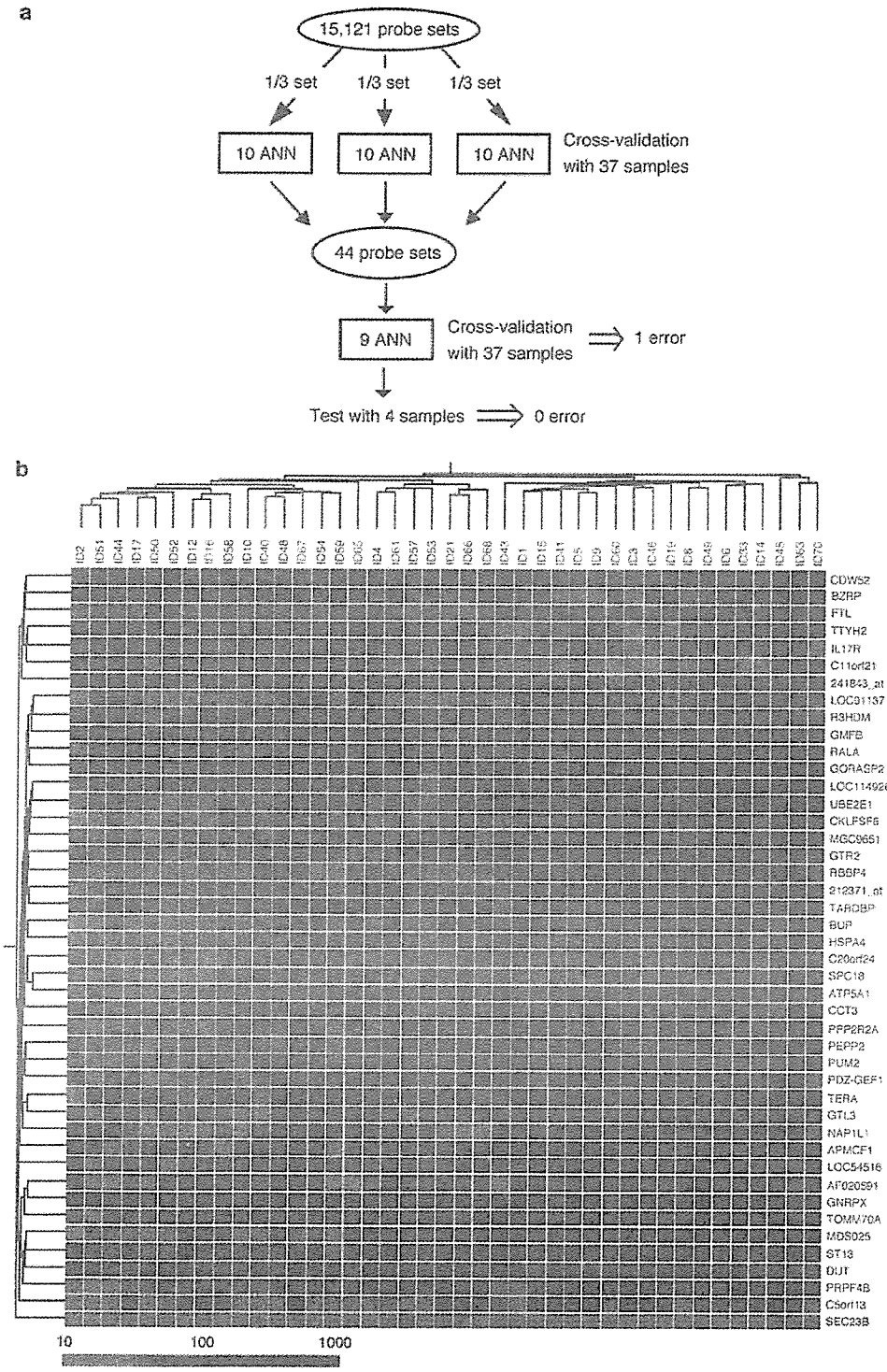

Figure 2 Schematic of the ANN analysis used for class prediction of ATL. (a) The 15 121 probe sets originally selected in Figure 1b were divided into three nonoverlapping groups, each of which was used as the input for 10 ANNs. We performed a 10% crossvalidation rotation with 37 samples, training with 33 samples and testing of the remaining four samples. On the basis of the differentiation process with the three sets of 10 ANNs, we selected 44 'predictor' genes whose expression markedly influenced the prediction accuracy in each set of ANNs. (b) Subject tree generated by two-way clustering analysis with the 44 predictor genes selected in (a) is demonstrated as in Figure 1c. 241843_at and 212371_at are the expressed sequence tag IDs designated by Affymetrix. Annotations and expression intensities for the probe sets are presented in Supplementary Table 3.

without such a gain ($P=0.0357$). These data indicate that gene dosage indeed affects transcript abundance in ATL cells. The large standard deviations apparent in the data shown in Figure 3e, however, suggest that other mechanisms (mediated by transcription factors or epigenetic regulation, for example) have also a large impact on gene expression level.

The hepatocyte growth factor-MET pathway in ATL cells

The long latency period for ATL in HTLV-I carriers suggests that the molecular pathogenesis of ATL and its stage progression might be highly heterogeneous. To identify molecular events that might contribute to transition to the acute stage, we next attempted to isolate 'acute stage-specific genes,' defined by their

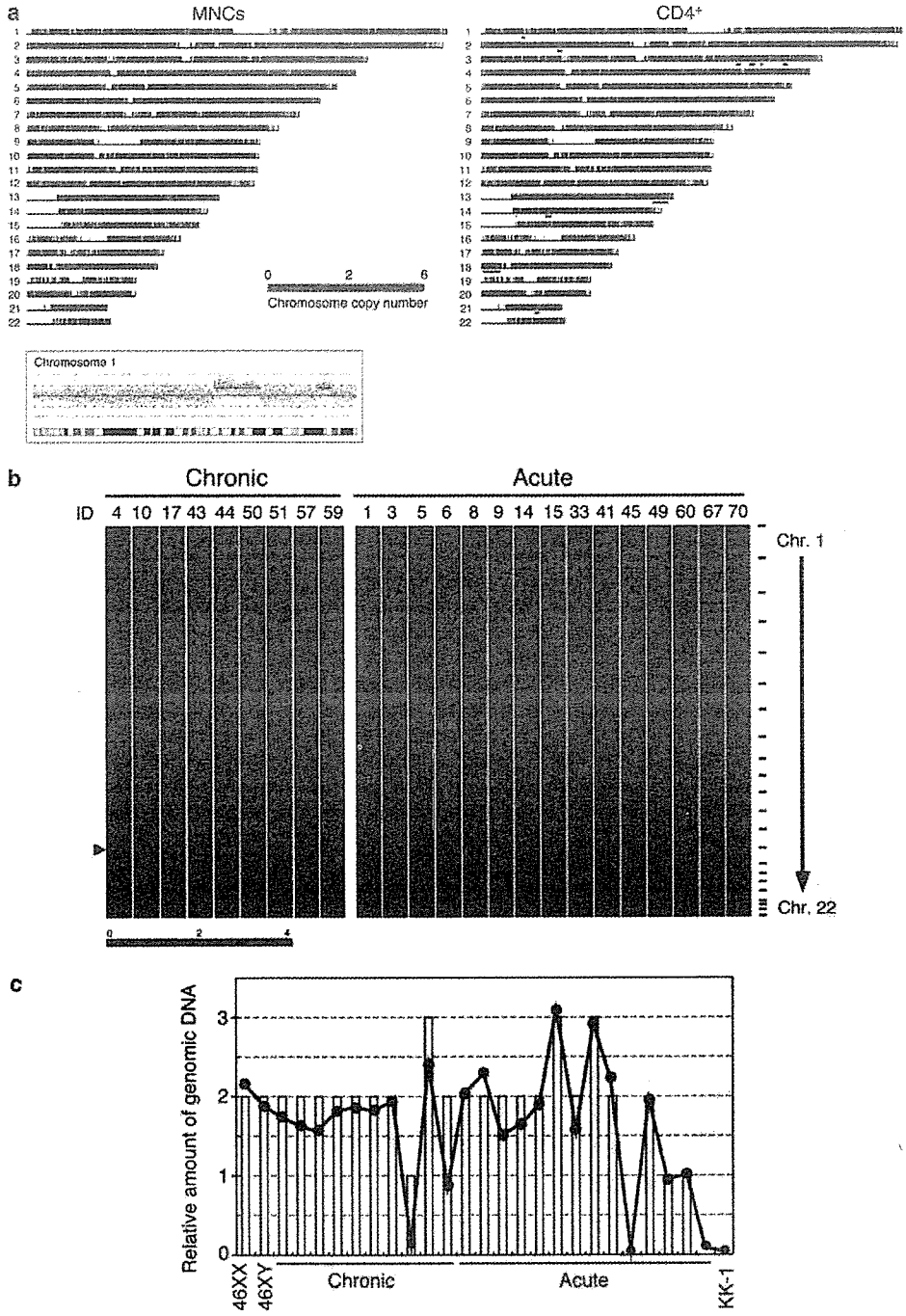

Figure 3 *Continued.*