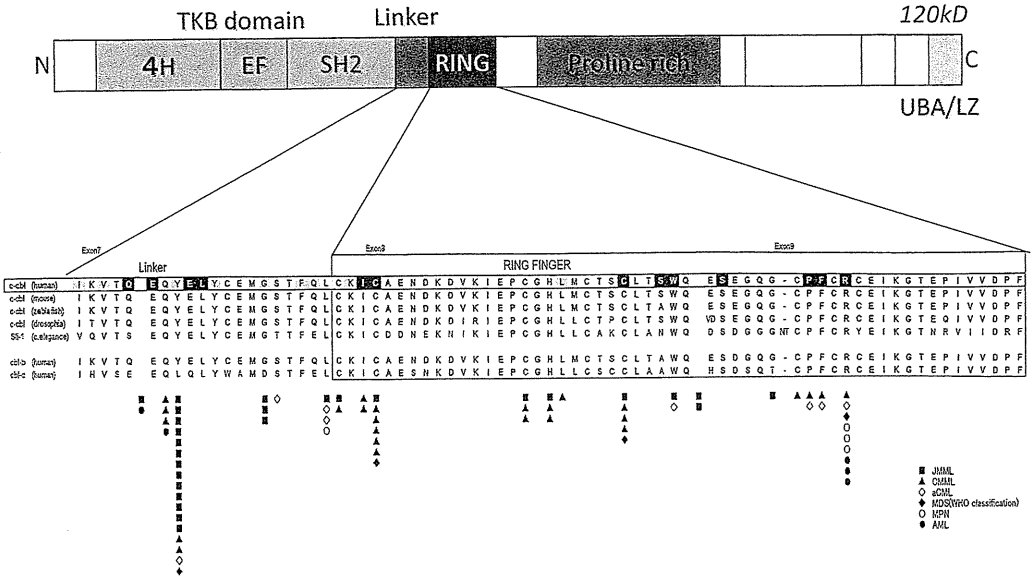


図2 c-CBLの構造と骨髄系腫瘍で観察されるCBL変異
c-CBLは，TKBドメイン，リンカー，RINGフィンガー・ドメイン，Proline-Richドメイン，UBA/LZドメインなどからなる。骨髄系腫瘍で同定されたc-CBL変異は，リンカーからRINGフィンガー・ドメインにかけて存在する。変異の多くは，E2ユビキチン結合酵素であるUbcH7との結合に重要とされるアミノ酸（黒・白抜き）やTKBドメインとの立体構造形成上重要なアミノ酸（灰）に生じており，これらのアミノ酸は種を越えて保存されている。リンカーからRINGフィンガーにかけての領域の欠失変異も報告されている。

れる等，骨髄増殖性疾患（myeloproliferative neoplasms, MPN）としての特徴も有していることから，WHO分類からMDS/MPNとして狭義のMDSから独立した疾患群である[24]。これまでの報告では，若年性骨髄単球性白血病（juvenile myelomonocytic leukemia, JMML）の10～17%[25,26]，CMMLの5～25%[7,16,27]，atypical chronic myeloid leukemiaの5%程度[17]に*c-CBL*変異は認められている。JMMLにおいては，腫瘍細胞特異的な後天的変異のみならず，胚細胞変異例も報告されている[28~30]。Noonan症候群はJMMLの合併が認められる先天性疾患であるが，これまでに原因遺伝子と知られている*PTPN11*，*SOS1*，*RAS*などの既知の遺伝子変異を有さないNoonan様の表現型を呈する症例の一部に*c-CBL*の胚細胞変異例があることが明らかとなっている。また*c-CBL*変異を有する家系も報告され[28]，遺伝的に*c-CBL*変異アレルをヘテロに有する者は心血管系の異常などを示すとともに，しばしばJMMLを併発する。*c-CBL*変異による家系内発症のJMMLにおいても，腫瘍細胞ではしばしば11q-aUPDによりホモ変異となるが，家系内発症例では時として自然寛解が認められることなどが特徴的である。CMMLでは，*RUNX1/AML1*変異や*TET2*変異，*JAK2V617F*変異を伴っている症例も観察される[7,27]。JMMLにおいては，*RAS*や*PTPN11*などRAS経路の変異とは排他的に認められるとされるが[25,26]，CMMLにおいては*RAS*変異との重複例も報告されている。

これまでに骨髄系腫瘍で報告されている*c-CBL*変異は，E3ユビキチンリガーゼとしての機能上重要であるE2との結合部位にあたるリンカーからRINGフィンガー・ドメインに集積をしており（図2），その多くは生物種を越えて高度に保存されているアミノ酸に生じている点突然変異である。JMMLにおける変異の大半はリンカードメインに存在する371番目のチロシンのアミノ酸置換であり[25,26]，AML例などでは，この領域内の部分欠失例も報告されている[7,17,31]。

変異c-CBLの分子機構

臨床検体で認められた変異*c-CBL*をマウスの上皮系細胞株であるNIH3T3細胞に遺伝子導入すると細胞は腫瘍化し[7,32]，変異c-CBLはtransforming能を有すると考えられる。またマウスの造血幹細胞に変異体を導入し，移植実験を行うと，移植されたマウスはMPNや全身性の肥満細胞腫を発症する[33]。その一方で*c-Cbl*を欠失させた遺伝子改変マウスは，慢性骨髄性白血病のモデルマウスである*BCR/ABL*遺伝子導入マウスと交配すると欠失マウスでは急性転化が促進される。また欠失マウ

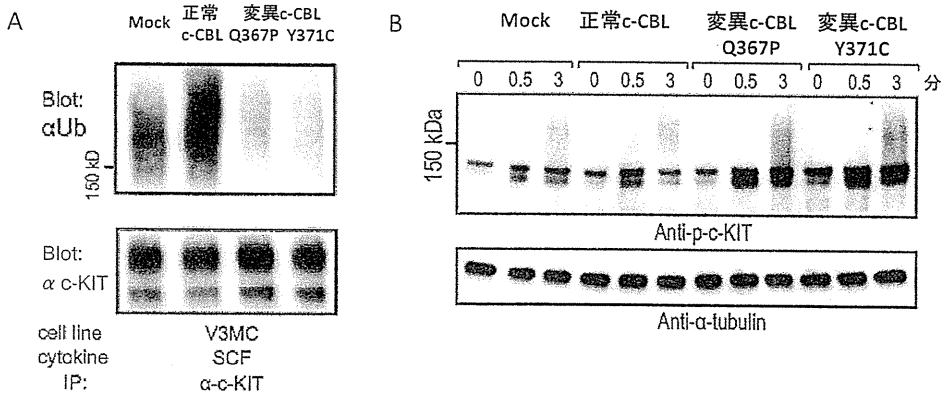
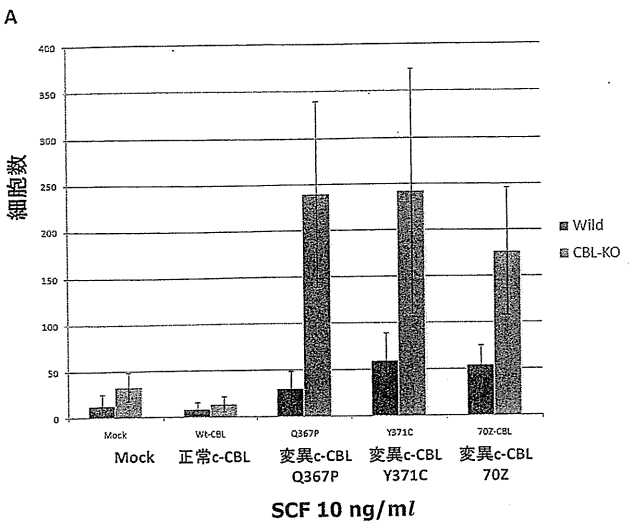


図３　変異 c-CBL とチロシンキナーゼのユビキチン化（文献７を改変）
変異 c-CBL をマウスの細胞株 V3MC（肥満細胞・c-Kit 発現細胞）に導入し，SCF 刺激を行うと，野生型 c-CBL 導入細胞では，速やかにユビキチン化を受けるが，変異体導入細胞ではユビキチン化が観察されない（A）。野生型 c-CBL 導入細胞では，SCF 刺激３分後にはリン酸化 Kit の発現量が低下するのに対し，変異体導入株では Kit のリン酸化状態の遷延が観察される。

スを長期に観察すると，すべての個体に特徴的な悪性腫瘍を発症することから，*c-Cbl* は，個体レベルではがん抑制遺伝子として機能していると考えられる[7]。すなわち，本変異においては，変異により *c-CBL* のがん抑制遺伝子としての機能が失われるのみならず，より積極的に腫瘍化へ導いていることが考えられる。

骨髄系腫瘍で観察される *c-CBL* 変異はユビキチンリガーゼとしての機能上重要なドメインに集積をするが，細胞株を用いた機能解析において，変異 c-CBL はユビキチンリガーゼとしての機能を喪失し（図 3A）[7, 17, 22]，変異体は正常 c-CBL のユビキチンリガーゼ作用を阻害する。その結果，変異 c-CBL 導入細胞において，サイトカイン刺激で活性化（リン酸化）されたチロシンキナーゼはユビキチン化を受けずに，リン酸化状態が遷延することが観察される（図 3B）。マウス造血前駆細胞（Lin-/Sca-1＋/c-Kit＋）に変異 *c-CBL* 導入すると，SCF（幹細胞因子）やトロンボポイエチンなどの広範な造血サイトカイン刺激に対し高感受性となり，過剰増殖が *in-vitro* で観察される。すなわち，サイトカイン刺激により活性化されたチロシンキナーゼがユビキチン化されないために，分解等を受けずに，細胞増殖シグナルが入り続けている効果と推測される。この現象は *c-Cbl* 欠失マウスの造血前駆細胞においても観察され，実際，c-Cbl 欠失マウスでは脾腫や造血前駆細胞の増加が個体レベルでも観察される[7, 34, 35]。変異 *c-CBL* 導入効果が単なるユビキチンリガーゼ機能の欠失や正常 c-CBL に対する阻害効果だけであれば，c-CBL が既に欠失した細胞には何の効果も示さないはずであるが，*c-Cbl* 欠失マウス由来の細胞に変異 c-CBL を導入すると，より顕著に観察される（図 4A）[7]。この結果から，変異 c-CBL は機能欠失変異であるとともに機能獲得型変異としても作用していると考えられる。このような現象は，代表的ながん抑制遺伝子である *p53* における一部の変異においても観察され，*p53* の相同遺伝子である *p63*，*p73* に対する阻害効果で説明がされている[36, 37]。c-CBL においても，CBL-B，CBL-C という相同性の高い分子が存在し[20]，こと CBL-B は血球系の細胞においても広く発現しているとされ，CBL-B に対する阻害効果で説明が可能かもしれない。実際，細胞株を用いた導入実験では変異 c-CBL は CBL-B のユビキチンリガーゼ機能も阻害することが確認されている[7]。また *c-Cbl* と *Cbl-b* の両者を欠失させたマウスは胎生致死であるが，骨髄でのみ条件的に両遺伝子を欠失させたマウスにおいては，重篤な骨髄増殖性疾患を発症することが報告された[38]。これらの観察結果は c-CBL 変異体による CBL-B に対する阻害効果が c-CBL 変異例における骨髄増殖性病態に関わっている可能性を支持する。

c-Cbl 欠失造血前駆細胞で観察される変異 c-CBL 導入によるサイトカイン高感受性は，正常c-CBLとの共導入により消失する（図 4B）[7]。この実験結果は，本異常においては変異が生ずるのみならず，正常*c-CBL*アレルが失われることも重要であることが示唆される。骨髄系腫瘍において観察される c-CBL 変異例の多くがヘテロ変異ではなく，11q-aUPD により正常 *c-CBL* アレルを欠失していることとも一致し，発がんにおける aUPD の寄与を考える上でも興味深いと思われる。



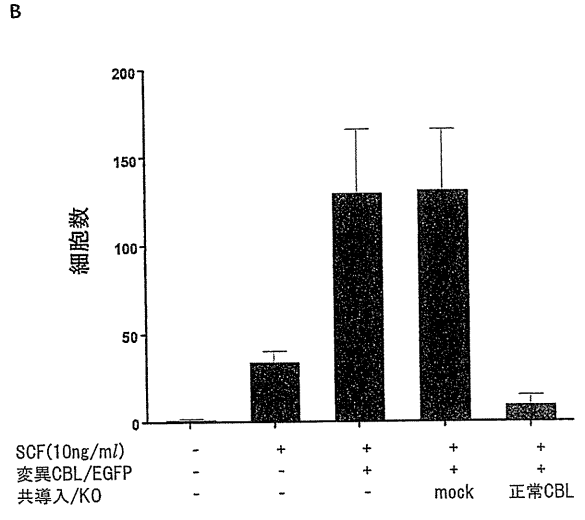


図4 変異 c-CBL によるサイトカイン高感受性（文献 7 を改変）
マウスの造血前駆細胞（Lin-/Sca-1+/c-Kit+）に正常または変異 CBL をレトロウィルスにより遺伝子導入し，サイトカインに対する感受性を細胞数で評価した。(A) *c-Cbl* 欠失（CBL-KO）マウスの造血前駆細胞は，SCF（幹細胞因子）刺激に対し，野生型（Wild）マウスに比して高感受性となり過剰増殖をする。変異 c-CBL 導入によっても過剰増殖は観察されるが，この効果は CBL-KO マウス由来の細胞おいてより顕著となる。同様の効果はトロンボポエチン，IL3，FLT3 リガンド刺激においても認められる。(B) 変異 c-CBL 導入により観察される c-Cbl 欠失造血前駆細胞のサイトカイン高感受性は正常 c-CBL との共導入により消失する。

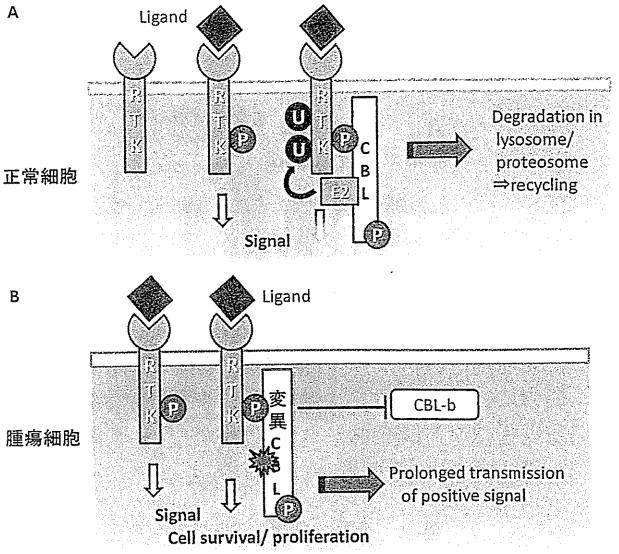


図5 CBL とチロシンキナーゼ・シグナル
(A) 正常 c-CBL は，サイトカインなどのリガンド刺激で活性化されたチロシンキナーゼ（RTK）に TKB ドメインで結合し，E2 ユビキチン結合酵素を介してチロシンキナーゼをユビキチン化する。ユビキチン化されたチロシンキナーゼ・c-CBL 複合体はプロテオソームやライソゾームで分解され，チロシンキナーゼを介したシグナルの重要な負の調整分子として機能する。(B) チロシンキナーゼの負の制御因子である c-CBL に変異が生じることにより，チロシンキナーゼはユビキチン化されずに活性化（リン酸化）状態が遷延し，シグナルが入り続けると考えられる。その結果として，CMML や JMML で認められる異常な血球増多が生じると推測される。変異 c-CBL はユビキチンリガーゼとしての機能を喪失するのみならず，CBL-b のユビキチンリガーゼ能をも阻害していると考えられる。

おわりに

これまでの知見から，*c-CBL* 変異を有する骨髄系腫瘍では，チロシンキナーゼの負の制御因子である c-CBL に変異が生じることにより，チロシンキナーゼを介した造血シグナルが入り続け，骨髄増殖性の病態が生じていると推測される（図 5）。発がんとチロシンキナーゼの異常の関連は，MPN や CML 以外にも，肺がん・乳がん・脳腫瘍・神経芽細胞種など幅広い腫瘍性疾患で示され，近年，多くのチロシンキナーゼ阻害剤が開発，臨床応用されている[39]。*c-CBL* 変異を有する骨髄系腫瘍における異常な血球増殖に対しても，チロシンキナーゼ・シグナルの制御が治療標的として有効であることが期待される。

SNP アレイによる網羅的なゲノム解析により同定された LOH/aUPD から骨髄系腫瘍における *c-CBL*，*TET2*，*EZH2* 変異が明らかとなったが，SNP アレイによる解析でも全く異常が観察されない症例が，典型的な MDS 例においても少なからず存在する。リシークエンス技術を代表とするゲノム解析技術は，ここ数年で革新的な進歩を遂げており，MDS 研究においても網羅的な

変異解析などを通じて，分子病態の理解が進み，臨床応用可能な治療標的分子が明らかとなることが期待される。

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LETTER TO THE EDITOR

Novel splicing-factor mutations in juvenile myelomonocytic leukemia

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Myelodysplastic syndromes (MDS) and myelodysplastic/myeloproliferative neoplasms (MDS/MPN) are heterogeneous groups of chronic myeloid neoplasms characterized by clonal hematopoiesis, varying degrees of cytopenia or myeloproliferative features with evidence of myelodysplasia and a propensity to acute myeloid leukemia (AML).[1] In recent years, a number of novel gene mutations, involving *TET2*, *ASXL1*, *DNMT3A*, *EZH2*, *IDH1/2*, and *c-CBL*, have been identified in adult cases of chronic myeloid neoplasms, which have contributed to our understanding of disease pathogenesis.[2-7] However, these mutations are rare in pediatric cases, with the exception of germline or somatic *c-CBL* mutations found in 10–15% of chronic myelomonocytic leukemia (CMML) and juvenile myelomonocytic leukemia (JMML),[8] highlighting the distinct pathogenesis of adult and pediatric neoplasms.[9]

Recently, we reported high frequencies of mutations, involving the RNA splicing machinery, that are largely specific to myeloid neoplasms, showing evidence of myeloid dysplasia in adult.[10] Affecting a total of eight components of the RNA splicing machinery (U2AF35, U2AF65, SF3A1, SF3B1, SRSF2, ZRSR2, SF1 and PRPF40B) commonly involved in the 3′ splice-site (3′SS) recognition, these pathway mutations are now implicated in the pathogenesis of myelodysplasia.[10] To investigate the role of the splicing-pathway mutations in the pathogenesis of pediatric myeloid malignancies, we have examined 165 pediatric cases with AML, MDS, chronic myeloid leukemia (CML) and JMML for mutations in the four major splicing factors, *U2AF35*, *ZRSR2*, *SRSF2*, and *SF3B1*, commonly mutated in adult cases.

Bone marrow or peripheral blood tumor specimens were obtained from 165 pediatric patients with various myeloid malignancies, including *de novo* AML ($n=93$), MDS ($n=28$), CML ($n=17$) and JMML ($n=27$), and the genomic DNA (gDNA) was subjected to mutation analysis (Supplementary Table 1). The status of the RAS pathway mutations for the current JMML series has been reported previously (Supplementary Table 2).[11,12] Nineteen leukemia cell lines derived from AML (YNH-1, ML-1, KASUMI-3, KG-1, HL60, inv-3, SN-1, NB4 and HEL), acute monocytic leukemia (THP-1, SCC-3, J-111, CTS, P31/FUJ, MOLM-13, IMS/MI and KOCL-48) and acute megakaryoblastic leukemia (CMS and CMY) were also analyzed for mutations. Peripheral blood gDNA from 60 healthy adult volunteers was used as controls. Informed consent was obtained from the patients and/or their parents and from the healthy volunteers. We previously showed that for *U2AF35*, *SRSF2* and *SF3B1*, most of the mutations in adult cases were observed in exons 2 and 7, exon 1, and exons 14 and 15, respectively.[10] Therefore, we confirmed mutation screening to these 'hot-spot' exons. In contrast, all the coding exons were examined for *ZRSR2*, because no mutational hot spots have been detected. Briefly, the relevant exons were amplified using PCR and mutations were examined by Sanger sequencing, as previously described.[10] The Fisher's exact test was used to evaluate the statistical significance of frequencies of mutations for *U2AF35*, *SF3B1*, *ZRSR2* or *SRSF2* in adult cases and pediatric cases. This study was approved by the Ethics Committee of the University of Tokyo (Approval number 948-7).

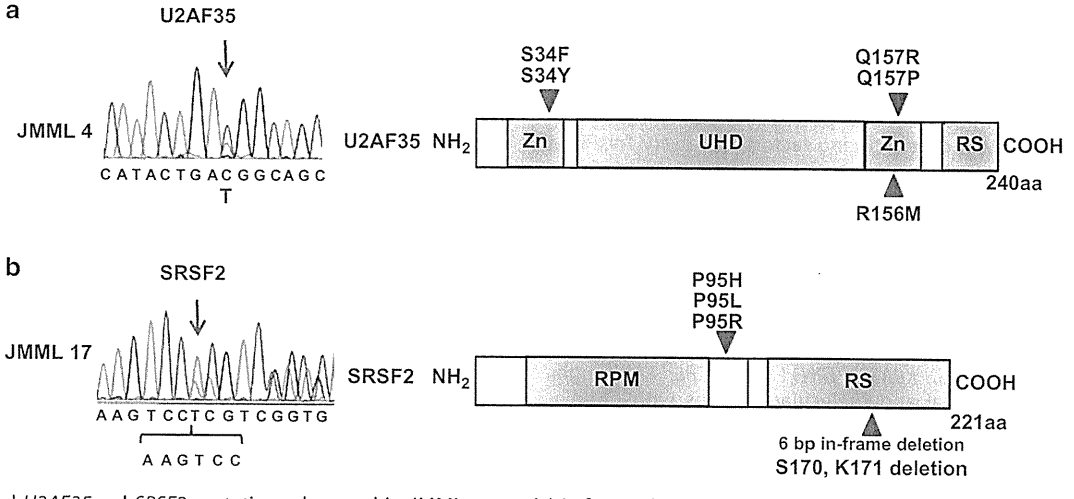

Figure 1. Novel *U2AF35*and *SRSF2* mutations detected in JMML cases. (**a**) Left panel: sequence chromatogram of a heterozygous mutation at R156 in N-terminal zinc-finger motifs of *U2AF35* detected in a JMML case (JMML 4) is shown. Mutated nucleotides are indicated by arrows. Right panel: illustration of functional domains and mutations of U2AF35. Red arrow heads indicate hot-spot mutations at S34 and Q157 detected in the adult cases.[10] Blue arrow head indicates the missense mutation at R156. (**b**) Left panel: sequence chromatogram of a 6-bp in-frame deletion (c.518-523delAAGTCC) in *SRSF2* detected in JMML 17 is shown. Mutated nucleotides are indicated by arrows. Right panel: illustration of functional domains and mutations of SRSF2. Red arrow head indicates hot-spot mutation at P95 frequently detected in the adult cases.[10] Blue arrow head indicates a 6-bp in-frame deletion leading to deletion of S170 and K171.

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No mutations were identified in the 28 cases with pediatric MDS, which included 13 cases with refractory anemia with excess blasts, 5 with refractory cytopenia of childhood, 2 with Down syndrome-related MDS, 2 with Fanconi anemia-related MDS, 2 with secondary MDS and 4 with unclassified MDS. Similarly, no mutations were detected in 93 cases with *de novo* AML or in 17 with CML, as well as 19 leukemia-derived cell lines. Our previous study in adult patients showed the frequency of mutations in *U2AF35*, *SF3B1*, *ZRSR2* or *SRSF2* to be 60/155 cases with MDS without increased ring sideroblasts and 8/151 *de novo* AML patients, emphasizing the rarity of these mutations in pediatric MDS ($P<5.0\times10^{-6}$) and AML ($P<0.02$) compared with adult cases. We found mutations in two JMML cases, JMML 4 and JMML 17. JMML 4 carried a heterozygous *U2AF35* mutation (R156M), whereas JMML 17 had a 6-bp in-frame deletion (c.518-523delAAGTCC) in *SRSF2* that resulted in deletion of amino acids S170 and K171 (Figure 1). Both nucleotide changes found in *U2AF35* and *SRSF2* were neither identified in the 60 healthy volunteers nor registered in the dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/) or in the 1000 genomes project, indicating that they represent novel spliceosome mutations in pediatric cases.

U2AF35 is the small subunit of the U2 auxiliary factor (U2AF), which binds an AG dinucleotide at the 3′SS, and has an essential role in RNA splicing.[13] With the exception of a single A26V mutation found in a case of refractory cytopenia with multilinage dysplasia, all the U2AF35 mutations reported in adult myeloid malignancies involved one of the two hot spots within the two zinc-finger domains, S34 and Q157, which are highly conserved across species, suggesting the gain-of-function mutations.[10] In JMML 4, the R156M U2AF35 mutation affects a conserved amino acid adjacent to Q157, suggesting it may also be a gain-of-function mutation, leading to aberrant pre-mRNA splicing possibly in a dominant fashion.

SRSF2, better known as SC35, is a member of the serine/arginine-rich (SR) family of proteins.[14] SRSF2 binds to a splicing-enhancer element in pre-mRNA and has a crucial role not only in constitutive and alternative pre-mRNA splicing but also in transcription elongation and genomic stability.[14] All mutations thus far identified in adult cases exclusively involved P95 within the intervening sequence between the N-terminal RNA-binding domain and the C-terminal RS domain.[10] This region interacts with other SR proteins, again suggesting that the P95 mutation may result in gain-of-function.[10] This proline residue is thought to determine the relative orientation of the two flanking domains of SRSF2, and a substitution at this position could compromise critical interactions with other splicing factors necessary for RNA splicing to take place. In contrast, the newly identified 6-bp in-frame deletion in JMLL 17 results in two conserved amino acids, S170 and K171, within the RS domain. Although it may affect protein–protein interactions, the functional significance of this deletion remains elusive.

JMML is a unique form of pediatric MDS/MPN characterized by activation of the RAS/mitogen-activated protein kinase signaling pathway; in 90% of cases, there are germ line and/or somatic mutations of *NF1*, *NRAS*, *KRAS*, *PTPN11* and *CBL*.[8] Although JMML shares some clinical and molecular features with CMML, its spectrum of gene mutations suggests that it is a neoplasm distinct from CMML.[15] This was also confirmed by the current results that the splicing-pathway mutations are rare in JMML, whereas they are extremely frequent (~60%) in CMML.[10] Although the two JMML cases carrying the splicing-pathway mutations had no known RAS-pathway mutations, both the pathway mutations frequently coexisted in CMML.[8]

To summarize, no mutations of *SF3B1*, *U2AF35*, *ZRSR2* or *SRSF2* are found in pediatric MDS and AML. In our study, except for *ZRSR2*, mutations were examined focusing on the reported hot spots in adult studies, raising a possibility that we may have missed some mutations occurring in other regions. However, these hot spots represent evolutionally conserved amino acids and have functional relevance, it is unlikely that the distribution of hot spots in children significantly differs from adult cases and as such, we could safely conclude that mutations of *SF3B1*, *U2AF35*, *ZRSR2* and *SRSF2* are rare in myeloid neoplasms in children. Finally, mutations of *U2AF35* and *SRSF2* may have some role in the pathogenesis of JMML, although further evaluations are required.

CONFLICT OF INTEREST

The authors declare no conflict interest.

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Single-Nucleotide Polymorphism Array Karyotyping in Clinical Practice: Where, When, and How?

Aiko Sato-Otsubo, Masashi Sanada, and Seishi Ogawa

Single-nucleotide polymorphism array (SNP-A) karyotyping is a new technology that has enabled genome-wide detection of genetic lesions in human cancers, including hematopoietic neoplasms. Taking advantage of very large numbers of allele-specific probes synthesized on microarrays at high density, copy number alterations as well as allelic imbalances can be sensitively detected in a genome-wide manner at unprecedented resolutions. Most importantly, SNP-A karyotyping represents the only platform currently available for genome-scale detection of copy neutral loss of heterozygosity (CN-LOH) or uniparental disomy (UPD), which is widely observed in cancer genomes. Although not applicable to detection of balanced translocations, which are commonly found in hematopoietic malignancies, SNP-A karyotyping technology complements and even outperforms conventional metaphase karyotyping, potentially allowing for more accurate genetic diagnosis of hematopoietic neoplasms in clinical practice. Here, we review the current status of SNP-A karyotyping and its application to hematopoietic neoplasms.
Semin Oncol 39:13-25

GENETIC ABNORMALITIES IN HEMATOLOGIC MALIGNANCIES

Since the discovery of the Philadelphia (Ph) chromosome in chronic myelogenous leukemia (CML) by Peter Nowell and David Hungerford in 1960,[1] hundreds of different genetic alterations/abnormalities have been identified and described in human cancers, including not only hematologic malignancies but also in a wide variety of solid cancers.[2] Human cancers show a diversity of genetic alterations, ranging from chromosome-scale lesions, such as translocations, gains/amplifications, and losses of large chromosomal segments, to small nucleotide substitutions, insertions, and deletions. Now it has been well established that the genetic alterations are central to the development of cancers, determining their biological or clinical behaviors. In fact, some genetic lesions, such as recurrent translocations, are highly specific to particular disease types or closely linked to tumor histologies, while others are commonly observed in a wide spectrum of cancer types, indicating more general roles of these genetic changes in carcinogenesis. Significantly, the information about these genetic lesions not only contributed to unmasking the underlying molecular pathogenesis of cancers but also enabled the development of novel diagnostics, therapeutics, and sensitive tumor monitoring that target these specific lesions.[3,4]

This has been best exemplified in hematologic cancers, in which underlying genetic changes have been most extensively studied."In particular, a number of disease-specific chromosomal translocations found in leukemias and lymphomas have been demonstrated to be critical genetic markers in clinical practice.[5-9] While several techniques have been developed to detect these genetic changes with different sensitivities and specificities for different purposes, probably the most widely used in clinical settings is metaphase karyotyping. The metaphase karyotyping technique was first developed during the 1960s and was soon introduced in both the experimental and clinical hematology fields. Since then, it has long been used as one of the indispensable clinical tests and research tools with which genetic alterations can be explored in a genome-

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wide fashion, although resolutions are limited. Nevertheless, recent advances in genomics and microarray technologies have provided a new and, in a sense, more powerful alternative: SNP-array (SNP-A)-based analysis of cancer genomes or SNP-A karyotyping.[10-12] Here, we will focus on this novel genetic approach to hematologic oncology.

BASIC PRINCIPLES OF SNP-A–BASED COPY NUMBER ANALYSIS OF CANCER GENOMES

SNP-As were originally developed for large-scale SNP typing to enable genome-wide association studies (GWAS), in which more than hundreds of thousands of common SNPs across the entire genome are genotyped for thousands of specimens.[13,14] Currently, two SNP-A platforms are commercially available, which achieve highly paralleled genotyping of more than a million SNPs relying on hybridization to, and/or extension from, allele-specific oligonucleotide probes synthesized in high-density on array matrix (Affymetrix, Santa Clara, CA, GeneChip SNP Genotyping Arrays)[13] or numerous micro-beads (Illumina, San Diego, CA),[14] respectively. While making tremendous contributions to the recent achievements through a number of GWAS studies[15-17] both SNP-A technologies also have been applied with excellent results to genome-wide copy number analysis of cancer genomes.[18-21]

For the purpose of genotyping, the relative intensities of the two kinds of SNP-specific signals at individual SNP loci are evaluated to discriminate three possible genotypes, such as A/A, A/B, and B/B (Figure 1a). On the other hand, for copy number analysis, these signals are compared across all SNP loci to calculate genome-wide copy numbers, using "reference signal values" for diploid DNA (SNP-A karyotyping)[10-12] (Figure 1b). Note that like other DNA-based analyses, SNP-A karyotyping cannot determine cell ploidy precisely, which can only be enabled by cell-based analysis[22] (for more detail, see Ogawa et al[22]). The basic idea here is similar to array-based comparative genomic hybridization (array CGH), in that the hybridization signals from tumor DNA are compared to normal diploid signals at individual probe sites.[23,24] However, in SNP-A karyotyping, the comparisons are made between the corresponding two SNP-specific probes, which makes it possible to calculate allele-specific copy numbers (AsCNs) (Figure 1b). The AsCN analysis is a unique feature of SNP-A-based copy number analysis,[25] enabling sensitive detection of copy neutral loss of heterozygosity (CN-LOH) or uniparental disomies (UPD), which cannot be detected by metaphase karyotyping or array CGH (see below).[25] In addition, their high resolutions of analysis to precisely point out genetic targets with their positions, high-throughput sample processing with semi-automated experimental procedures, and cell division-independent nature of analysis, as well as computer-based detection of genetic lesions, are also among the outstanding features of SNP-A karyotyping platforms compared to conventional metaphase karyotyping and array CGH.

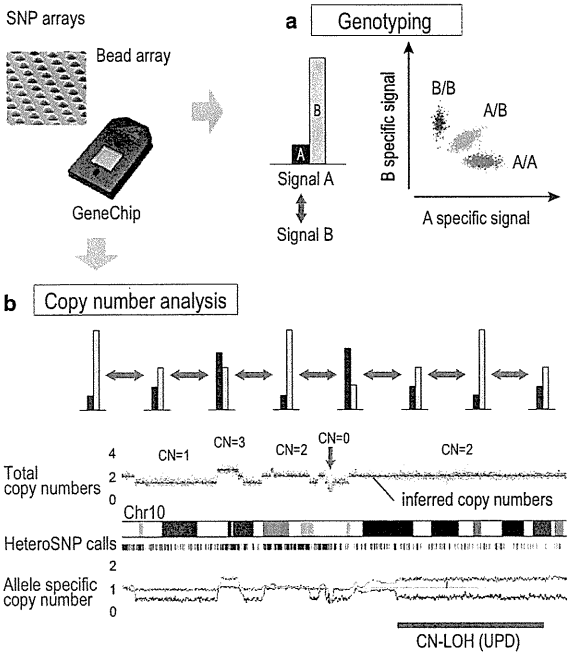

Figure 1. Principles of genotyping and copy number detection in SNP-A platforms. SNP arrays were originally designed for large-scale SNP typing, in which relative intensity of SNP-specific signals at each SNP site is compared to discriminate among three possible genotypes (a). On the other hand, SNP-specific signals can be used to calculate allele-specific copy numbers (AsCNs) by comparing them across the genomes in an allele-specific manner (SNP-A karyotyping). (b) A typical result of SNP-A karyotyping generated by CNAG software, where AsCNs are shown below the chromatogram (red and green lines), together with total genomic copy number plots on the top panel. The right end of the chromosome segment shows copy neutral LOH or UPD, as indicated by dissociated AsCN graphs with the normal total copy number (n = 2).

THE TARGET GENETIC LESIONS

In principle, the targets of SNP-A karyotyping are strictly limited to those genetic lesions that cause copy number alterations, such as numerical abnormalities of chromosomes and gains or losses of chromosomal segments. Balanced translocations, which are commonly found in hematopoietic malignancies and would be easily detected by metaphase karyotyping, such as t(8;21)(q22;q22) and t(15;17)(q22;q21), are not accompanied by copy number changes and therefore are out of scope of the SNP-A karyotyping. Moreover, SNP-A karyotyping does not provide any topological information about the copy number abnormalities it detects. For example, a high-level gene amplification is a very

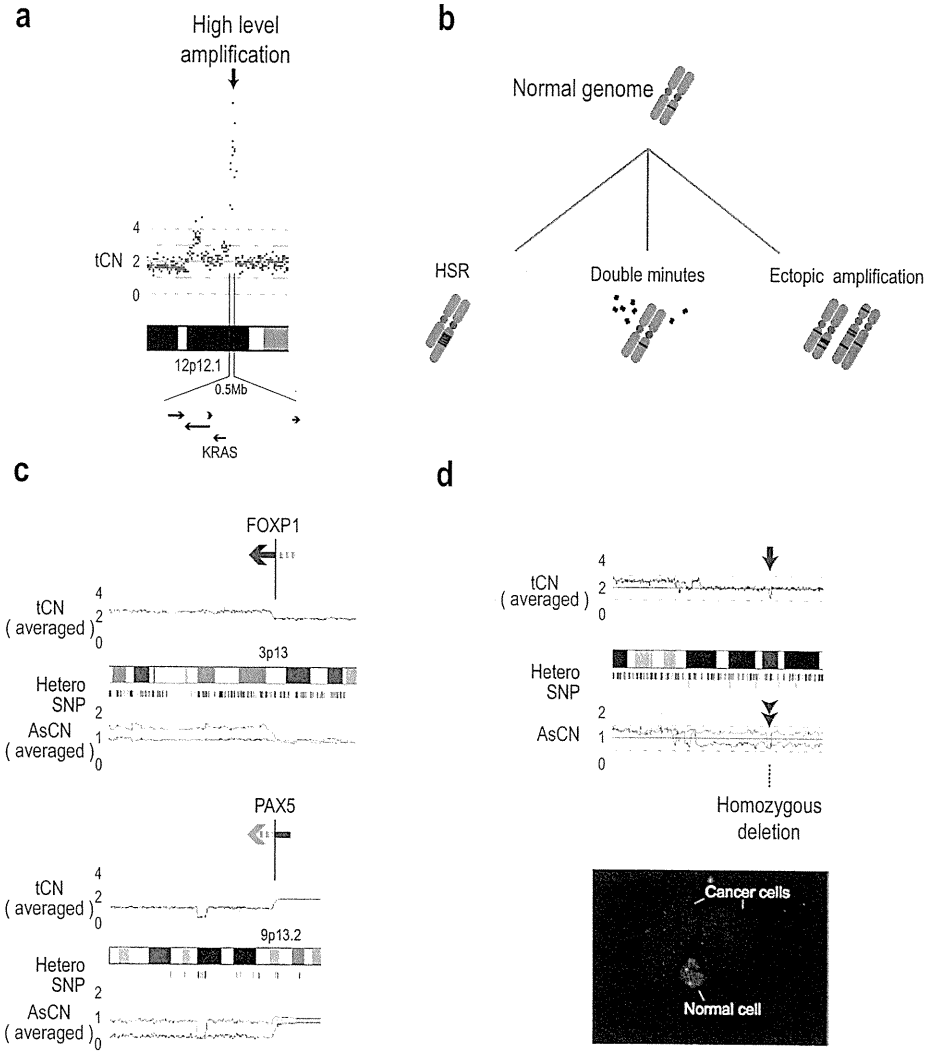

Figure 2. Detection of high-level amplifications and homozygous deletions in SNP-A karyotyping. (a) SNP-A karyotyping sensitively detects focal gene (chromosome) amplifications involving KRAS locus in a MDS case. (b) Gene amplifications may occur in situ as homogeneously staining regions (HSR), in episomal sites as double minutes (DMs), or at ectopic chromosomal sites. However, SNP-A karyotyping does not provide topological information of the amplifications. (c) SNP-A analysis of an unbalanced translocation between chromosomes 3 and 9 causing a *FOXP1/PAX5* fusion gene. The genomic positions of the breakpoints are determined at high precision. (d) Output of SNP-A karyotyping showing a homozygous deletion at 1p21.3 in a lymphoma specimen. The focal reduction found in total copy number plot (red arrows) is judged to represent a homozygous deletion, based on the fact that the region shows a biallelic reduction of AsCNs, as indicated by arrowheads.

nice target of SNP-A karyotyping, but it does not determine where and how it occurs within the genome, or whether it presents in a homogeneously stained region (HSR) or double minutes (DM), or represents episomal gene amplification (Figure 2a and b). On the other hand, in SNP-A karyotyping copy number change breakpoints can be precisely determined, relying on the method's high level of resolution (Figure 2a).

In terms of resolution, the SNP-A platforms far outperform metaphase karyotyping and typical bacterial artificial chromosome (BAC) array-based CGH, although oligonucleotide-based CGH arrays (Agilent, Santa Clara, CA) show even better performance in this regard.[26] On the currently available SNP arrays, genomic copy numbers are measured at approximately 10^5 to $>10^6$ SNP loci (Affymetrix GeneChip and Illumina BeadChip). Generally, the behavior of individual SNP-specific probes is not reliable enough to allow for precise single-point determination of copy number alterations at each SNP site, but the use of large numbers of probes enables the detection of genetic lesions less than 100k in size that would easily escape detection by metaphase karyotyping or even by BAC array CGH. SNP-A can detect the genes involved in the breakpoints of the unbalanced copy number changes[20,27] and precisely determine the genetic targets of amplifications and deletions[18,20,28-30] (Figure 2c).

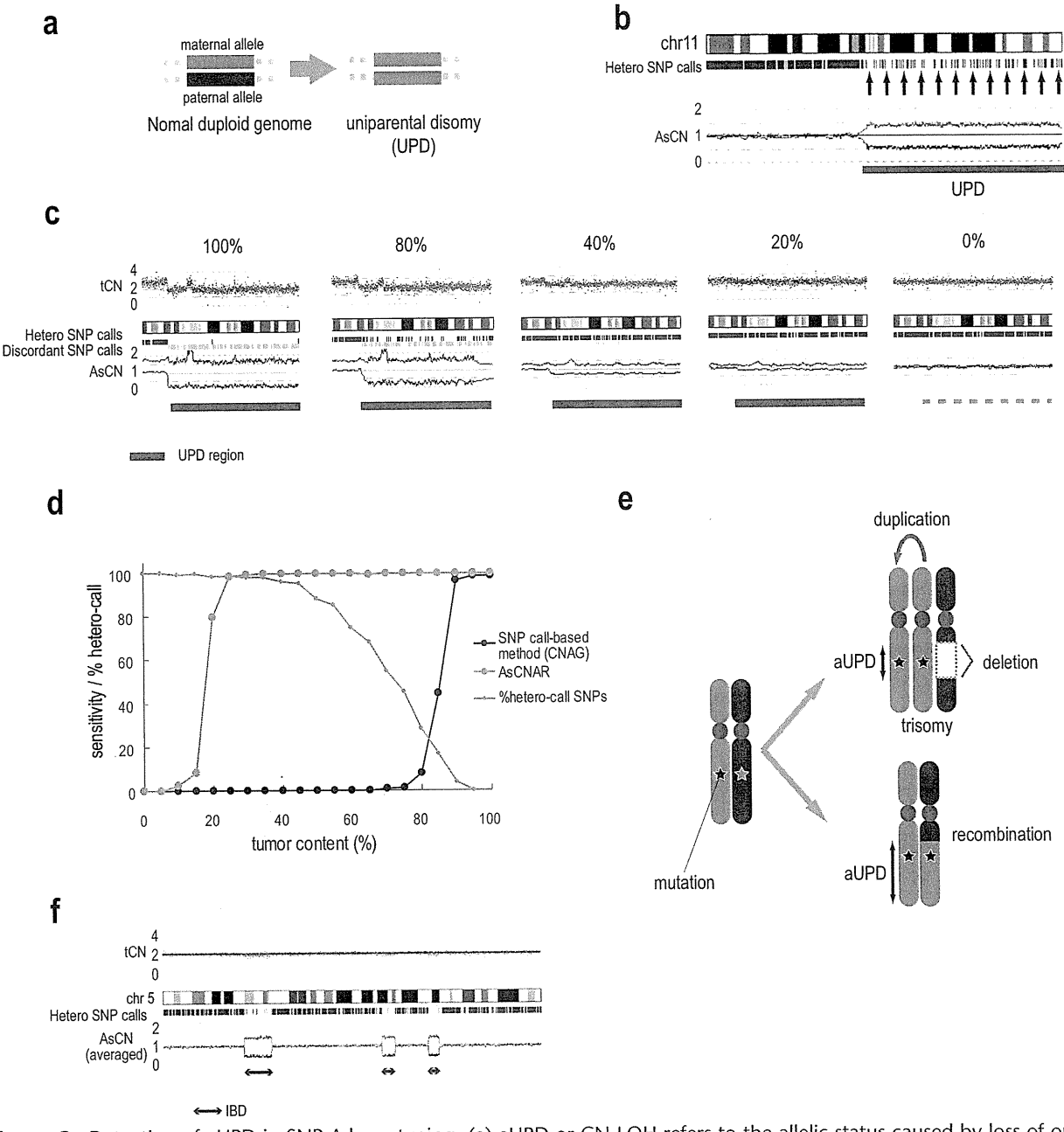

Figure 3. Detection of aUPD in SNP-A karyotyping. (a) aUPD or CN-LOH refers to the allelic status caused by loss of one of the two parental alleles and duplication of the remaining allele. (b) In SNP-A karyotyping, aUPD is detected by significant dissociation of AsCNs (red arrows) or by the reduction of the number of heterozygous SNP calls (blue arrow). (c) Sensitive detection of aUPD using AsCN analysis was evaluated using intentionally mixed tumor and normal cells at the indicated tumor proportions. The reduction of heterozygous SNP calles (green bars) in the aUPD(+) region is obscured with less than 40% of tumor content, whereas the dissociation of AsCNs (green and red lines) clearly indicates the presence of aUPD even with. 20% of tumor content. (d) AsCN-based detection of aUPD (orange) outperforms that relying on the reduced heterozygous SNP calls (blue) in sensitivity. The gray line indicates numbers of heterozygous SNP calls within the target region with aUPD. (e) aUPD is generated as a result of somatic recombination between sister chromatids or deletion of a chromosome segment and duplication of the remaining allele, rendering a mutated allele homozygous. (e) Disappearance of heterozygous SNP calls are also caused by inheritance of identical IBD alleles from parents. Reflecting multiple meiotic recombinations within the parents' gametes, they usually appear as multiple segments with loss of heterozygous SNP calls intervened with heterozygous diploid segments.

Homozygous deletions are less common than simple deletions in cancer genomes, but they provide an important clue to the identification of tumor-suppressor genes, since the typical size of homozygous deletions is less than 1 Mb. Taking advantage of their high resolution of analysis, the SNP-A platforms enable genome-wide detection of these lesions and have contributed to the discovery of novel tumor-suppressor genes.[29,30] While in primary tumor specimens, the presence of normal cells frequently prevents detection of homozygous deletions by polymerase chain reaction (PCR), such deletions could be detected as a biallelic reduction in AsCNs in SNP-A karyotyping even with low tumor contents (Figure 2d).

DETECTION OF CN-LOH OR UPD

The other target of SNP-A karyotyping is CN-LOH. CN-LOH has been the focus of recent attention in cancer genetics, especially hematopoietic neoplasms. It represents an abnormal allelic status, in which both of the two existing alleles have a single parental origin, and thus it is also called "uniparental" disomy (UPD) (Figure 3a). In SNP-A karyotyping, UPD is detected as a significant dissociation in AsCN plots, where higher and lower copy number plots indicate the duplicated and missing alleles, respectively (Figure 3b). When the proportion of UPD-positive tumor components exceed 70% in the specimen, the frequency of heterozygous SNP calls is significantly reduced, indicating the presence of LOH. However, detection of LOH relying on heterozygous SNP calls is much less sensitive compared to the AsCN-based detection; with less than 70% of UPD-positive tumor cells, no significant reduction of heterozygous SNP calls is observed (Figure 3c). Although the size of the dissociation in AsCN plots varies depending on the proportion of the tumor components having UPD within the specimen, as few as 20% of UPD-positive components can be detected by SNP-A karyotyping[25] (Figure 3d).

UPD may occur as an inborn error in congenital disorders, including Beckwith-Wiedemann syndrome (UPD in 11p), and Angelman syndrome and Prader-Willi syndrome (UPD in 15q), where the consequent abnormal imprinting status of the involved chromosomes is implicated in their pathogenesis.[31-33] However, recent studies using SNP-A karyotyping indicate that UPD is more commonly found in cancers as an acquired abnormality (acquired UPD [aUPD]).[34] Several mechanisms have been implicated in the generation of aUPD during the development of cancer (Figure 3e). For example, mis-segregation of a chromosome with total or partial deletion of the other allele is thought to be a common mechanism of aUPD among cancers, especially those showing hyperploidy, leading to aUPD of whole chromosomes or aUPD plus trisomy of the surrounding chromosomal segments. On the other hand, in many hematopoietic neoplasms aUPDs frequently involve the telomere end of affected chromosomal arms, suggesting that they are generated by somatic recombinations between sister chromatids. These aUPDs should be strictly discriminated from identity-by-descent (IBD) alleles, which are not uniparental but inherited from both parents by varying degrees of consanguinity between close kin. For example, one sixteenth of the total genome is expected to consist of IBD alleles in children born to marriage between cousins. Thus, IBD alleles are more common in older individuals, reflecting higher frequencies of consanguinity in the past. Usually, IBD alleles tend to be found in the middle of diploid regions and involve multiple chromosomal sites (Figure 3f).[35,36] Unfortunately, however, discrimination between aUPD and IBD alleles is difficult in some cases, especially when they occur in mostly diploid genome and involved chromosomal ends.

In cancer genetics, aUPD has been established as one of the common mechanisms for biallelic inactivation of tumor-suppressor genes, by which the intact allele is lost and replaced by the mutant allele.[37] However, the precise incidence of aUPD among human cancers has not been fully evaluated until recently, when the genome-wide detection of this abnormality has been enabled by the advent of the SNP-A karyotyping technology. aUPD has been shown to frequently occur in human cancers, including hematopoietic neoplasms. aUPDs are found in 20% of acute myeloid leukemia (AML), 30% of myelodysplastic syndromes (MDS), and related disorders, and more than 80% of malignant lymphomas.[30,35,38-45] As expected, these UPDs are shown to be tightly associated with homozygous mutations of known tumor-suppressor genes, including *TET2* in 4q, *CDKN2A/B* in 9p, *TP53* in 17p, *NF1* in 17q, *Rb* in 13q, *CEBPA* in 19q, and *RUNX1* in 21q[28,35,37,40-42,44] (Figure 4a). Moreover, recent evidence suggests that aUPD may accompany not only loss-of-function alleles of tumor-suppressor genes but also gain-of-function alleles of oncogenes. This was first demonstrated for 9pUPD causing homozygous *JAK2* V617F mutations in polycythemia vera (PV), as well as other myeloproliferative neoplasms (MPN), and to a lesser extent in MDS.[46-48] Thereafter, the association between aUPDs and oncogenic mutations was further confirmed for oncogenes in a variety of hematopoietic neoplasms.[35,49-51] Common examples include homozygous mutations of *c-MPL* or *NRAS*, *c-CBL*, and *FLT3*, which are caused by aUPDs in 1p, 4q, 7q, 11q, and 13q, in a variety of myeloid neoplasms, respectively (Figure 4b).

SENSITIVITY OF SNP-A KARYOTYPING

The sensitivity to detect particular genetic lesions in SNP-A karyotyping depends on the size of genetic le-

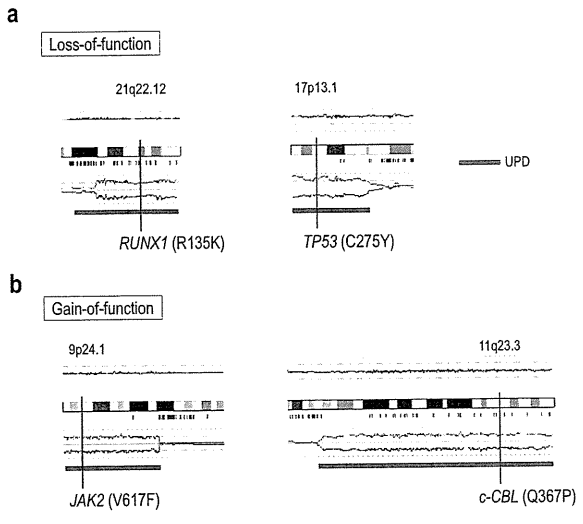

Figure 4. Recurrent aUPDs and their gene targets in hematopoietic neoplasms. Recurrent aUPD found in hematopoietic neoplasms are frequently associated with loss-of-function mutations of tumor-suppressor genes (a) or gain-of-function oncogenic mutations (b).

sions and the tumor contents within the samples, and also on the algorithm with which they are detected. These computer-assisted algorithms, as well as other bioinformatics tools for SNP-A karyotyping, are especially useful to detect complex genetic lesions objectively and to summarize them for a large number of specimens, facilitating identification of genetic targets. A number of algorithms for computer-assisted detection/inference of genetic lesions in SNP-A karyotyping have been developed, among which hidden Markov model (HMM)-based algorithms and those using circular binary segmentation (CBS) are widely applied by researchers.[52-54] Regardless of algorithms, to detect copy number changes the size of the measured copy number changes (Δ) needs to be significantly larger than the mean size of measurement errors, eg, standard deviation of measured copy numbers in diploid genome (SD^{dip}). Because the relative intensity of probe-specific signals to the background signals is substantially weaker in Affymetrix GeneChip than in CGH arrays, the mean log2 ratio of haploid to diploid signals remains approximately 0.5 (Nsp250K arrays) rather than achieves the theoretically expected value ($\sim$1.0) obtained in CGH arrays. In addition, SNP-A tends to show significantly higher SD^{dip} values than array CGH systems. As a result, SNP array-based copy number detection is more prone to loss of sensitivity with low tumor cell components than CGH-based copy number analysis. In typical SNP-A analyses, approximately 20% to 30% of tumor contents are required for detection of abnormalities in large chromosomal segments. In contrast, similar genetic lesions could be successfully captured even with less than 10% of tumor contents in metaphase karyotyping and typical CGH arrays (BAC array and Agilent 224K), although metaphase karyotyping depends on viable cells capable of cell division for analysis.

The SD^{dip} values or noises in SNP-A show substantial variation depending on the experimental conditions and the algorithms with which copy numbers are calculated. In the Affymetrix platform, the genomic DNA is digested with a proper restriction enzyme and the adapter-ligated restriction fragments are subjected to PCR amplification before hybridization. Because PCR amplification assumes successful digestion of both ends of the fragments, the difference in the mean length of genomic DNA between test and reference DNA can bias copy number calculation, especially at those SNP sites on the longer DNA fragments. This causes a serious problem to analyze degraded DNA prepared from formalin-fixed paraffin-embedded (FFPE) samples, although the problem is partly circumvented at the cost of resolution by eliminating SNPs on long restriction fragments (>500 bp) from the analysis.[55] The subsequent PCR reactions also produce biases, because relative amplification efficiency among different DNA fragments could be easily affected by subtle differences in PCR conditions, including types of polymerase and thermal cyclers.[12,56] Thus, in order to obtain the best results, it is very important to perform experiments as uniformly as possible between test and reference samples. For example, it is recommended that whenever possible, array experiments should be performed with a set of normal DNA included for reference, especially in those centers with less experience in SNP-A analysis, although this leads to increased costs and reduced throughput. Using a set of array data from normal DNA as a common reference can reduce costs and increase throughput but generally results in increased SD^{dip} and reduced resolution and sensitivity (Figure 5). SD^{dip} values in typical experiments are between 0.15 and 0.20, while they can be controlled to less than 0.10 in well-performed experiments.

COPY NUMBER VARIATIONS AND THE USE OF GERMLINE CONTROL

Copy number variations (CNVs) are a type of polymorphism widely found in our genomes, where the number of particular genomic segments shows variations.[57-59] Most CNVs are less than 1 Mb in length, but some CNVs span genomic segments of more than several megabases in length. While CNVs could be potential targets of SNP-A karyotyping, they may complicate the discrimination between somatic and germline events in cancer specimens, because difference in CNVs between test and references from different individuals could be erroneously detected as somatic copy number changes. Although using a germline DNA as a reference could largely circumvent the false positive

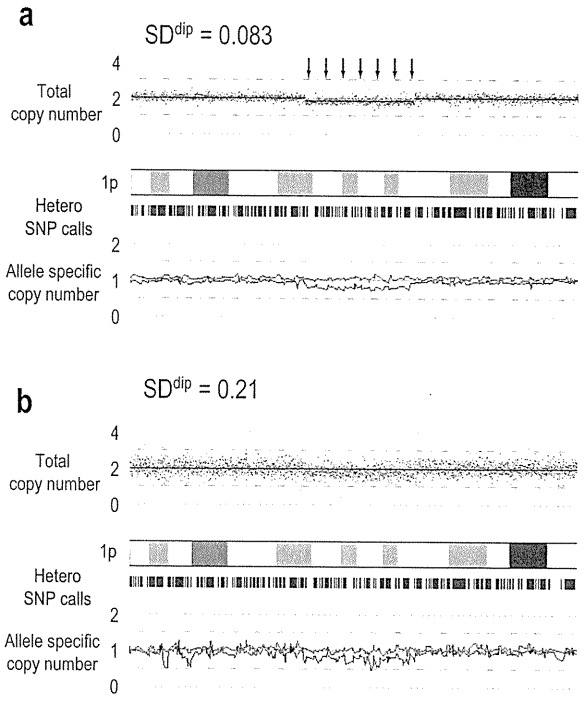

Figure 5. Effect of reference sets on detection of genetic lesions. The same array data for a tumor sample was analyzed with a set of reference array data of simultaneously processed normal diploid DNAs (a) or with a different array data set of normal diploid DNAs generated from a different set of experiments (b). The set of reference used for the analysis substantially influenced the result. The analysis in a shows a lower SD^{dip} value than that in (b), enabling identification of a interstitial deletion (red arrows) that is not clear in (b).

detection of CNVs as somatic copy number changes, it does not completely avoid the problem. When a CNV is located within a segment showing an allelic imbalance and analyzed with a germline control, an apparent copy number change may appear at that CNV locus, even though it is not real (Figure 6).

CLINICAL APPLICATIONS

Until recently, application of SNP-A karyotyping has been largely limited to exploratory research on cancer genetics. However, given its excellent performance in detecting genetic abnormalities in cancers, application of SNP-A karyotyping to clinical hematology could be a logical approach in an attempt to establish better management of cancer patients, although there remains a number of issues to be answered before its use in clinical settings. Clearly, SNP-A karyotyping does not replace the conventional metaphase karyotyping or other PCR-based detection of a variety of fusion genes, because SNP-A karyotyping cannot detect balanced translocations that are relevant to the management of a variety of hematopoietic malignancies.

Give their primary use for GWAS studies, processing a large number of specimens is an important pre-requisite for the development of SNP-A platforms. With simplified experimental protocols and semi-automated procedures, both SNP-A platforms achieve high-throughput sample processing, in which dozens of specimens can be analyzed within a few days in a single set of SNP array systems. This is in contrast to conventional metaphase karyotyping. Obtaining high-quality metaphases may not always be possible and, as previously mentioned, absolutely requires cell culture before analysis, precluding the analysis of archived samples. Production of a large enough number of karyograms for analysis is also time-consuming and their interpretation requires some discipline.

On the other hand, metaphase karyotyping may reveal the presence of several tumor subpopulations with different genomic profiles, as typically found in some MDS or AML M6 patients with poor prognosis, where individual metaphases show different karyotypes. DNA-based analyses including SNP-A and CGH array measure mean copy numbers among different subclones. They could infer such complexities in some cases, but generally would fail to fully dissect such complex abnormalities within each tumor subpopulation, suggesting the importance of combined used of metaphase karyotyping and array-based karyotyping technologies. Features of different platforms for detection of genetic alterations are summarized in Table 1. Apparently, what is important is the judicious use of the appropriate platforms according to the types of target genetic lesions to be detected.

As long as the target genetic abnormalities are un-

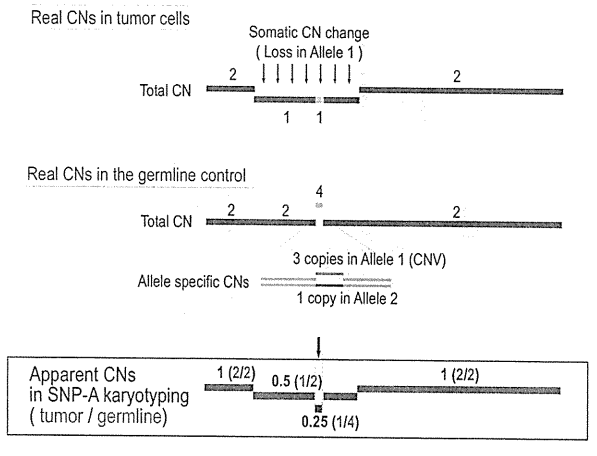

Figure 6. False detection of copy number abnormality. In most cases, CNVs are successfully discriminated from somatic changes using a germline control. However, in some cases, the use of a germline control may lead to false detection of CNVs as somatic changes. This occurs when two parental alleles have different CNVs and that CNV site is located in a segment showing copy number gain or loss.

Table 1. Comparison of Different Platforms

	Resolution	Mean Probe Interval	Dependence on Cell Division	Genome Coverage	Detection of UPD	Detection of Tumor Subclones	Sensitivity to Tumor Components	Detection of Balanced Translocation	Throughput	Cost
Cytogenetics	Low	>5–10Mb	+	+	−	++	~10%	+	Low	Low
Interphase FISH	NA	NA	−	−	−	NA	<~5%	+***	Intermediate	Low
aCGH	High	1.8kb*~70kb	−	++	−	+/−	10–20%	−	Intermediate	High
SNP-A	High	5kb**~20kb	−	++	+	+/−	20–30%	−	High	High

*Agilent 1M.
**Affymetrix SNP6.0.
***Selected targets only.

balanced changes shared by the main tumor population, SNP-A karyotyping would be a good alternative to conventional karyotyping or could even outperform the latter, especially when no metaphases are obtained in conventional karyotyping. SNP-A karyotyping reveals comprehensive registries of genetic lesions, including copy number gains and losses, as well as UPD, in hematologic neoplasms (Figure 7). In hematopoietic neoplasms, aUPDs are found in varying frequencies depending on tumor types, ranging from approximately 20% in AML to nearly 80% in diffuse large B-cell lymphoma. While some aUPDs are closely related to particular gene mutations, the clinical significance of other aUPDs remains to be elucidated.

AML AND ACUTE LYMPHOBLASTIC LEUKEMIA

In leukemias and lymphomas, a number of novel genetic targets have been identified through SNP-A karyotyping of acute lymphoblastic leukemia (ALL). SNP-A karyotyping identified recurrent deletions/translocations involving *EBF1* and *PAX5* in childhood ALL,[20,27,28] and frequent deletion of *Ikaros* in lymphoid blastic crisis of CML, as well as Ph1$^+$ ALL.[29] Meanwhile, the clinically relevant disease-specific translocations are out of the scope of SNP-A karyotyping, which are among common targets in metaphase karyotyping and could be more sensitively detected by targeted approach, including interphase fluroesence in situ hybridization (FISH) and reverse transcriptase-PCR. This is a major drawback of SNP-A karyotyping. However, the excellent performance of SNP-A karyotyping in genome-wide detection of complex unbalanced lesions as well as aUPD could compensate the drawback, and add unique values to this platform in clinical setting.

MDS AND RELATED MYELOID NEOPLASMS

MDS, MDS/MPN, and secondary AML are among the best targets of SNP-A karyotyping, in which the unbalanced genetic changes are predominant,[60,61] and these changes are directly incorporated into their prognostic scores.[62,63] It was demonstrated that SNP-A karyotyping showed a higher performance compared to metaphase karyotyping.[35,43] In our series consisting of 222 cases with MDS and related myeloid neoplasms, SNP-A karyotyping captured all the genetic lesions found in metaphase karyotyping except for four balanced translocations. Moreover, 41 of the 91 cases with normal karyotypes by metaphase cytogenetics showed one or more genetic lesions by SNP-A karyotyping. Overall, SNP-A karyotyping revealed approximately 1.5 times more genetic lesions, including -7/7q- and complex karyotypes indicating poor prognosis[35,43] (Figure 8). Assuming that the masked lesions in metaphase karyotyping are also valid in evaluating the International Prognostic Scoring System (IPSS) score,

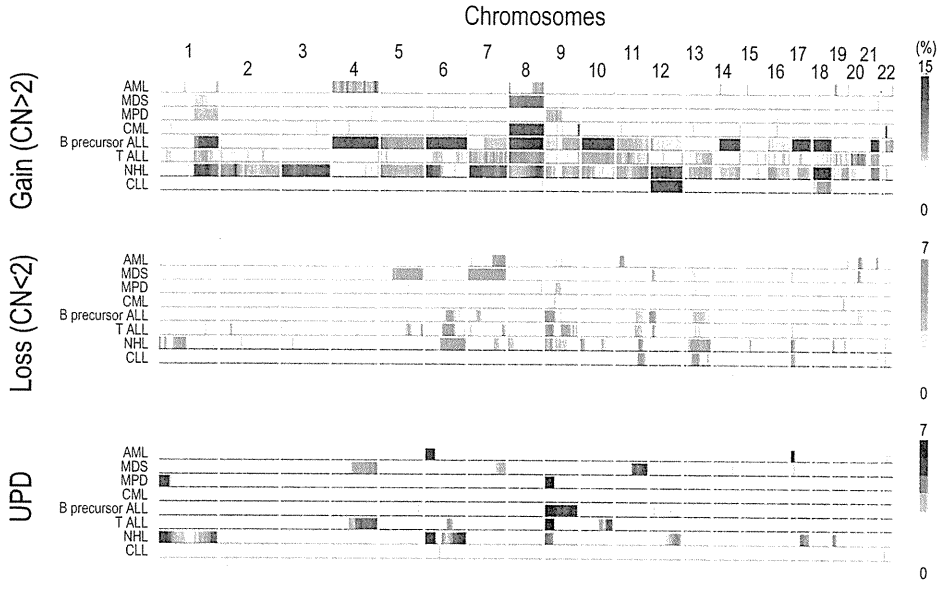

Figure 7. Genomic profiles of different hematopoietic neoplasms in SNP-A karyotyping. Genomic profiles revealed by SNP-A karyotyping are shown for different hematopoietic neoplasms, including AML (N = 36), MDS (N = 294), MPN (N = 57), CML (N = 51), B-precursor ALL (N = 507), T-cell ALL (N = 84), non-Hodgkin lymphoma (NHL) (N = 238), and CLL (N = 131). Frequencies of copy number gains and losses, as well as aUPDs, across the genome are color-coded in each neoplasm type as indicated. Each neoplasm type has a characteristic genomic profile of its own.

SNP-A karyotyping would be a more appropriate tool for the management of MDS and related neoplasms.

In these myeloid neoplasms, aUPDs are found in about one fourth to one third of the patients and, in some cases, represent the only genetic lesions found by SNP-A karyotyping.[35,43] These aUPDs are preferentially involved in particular chromosomal arms, such as 1p, 1q, 4q, 7q, 9p, 11p, 11q, 13q, 14q, 17p, and 21q. Importantly, recent studies demonstrated that many of these aUPDs are tightly associated with mutations of tumor-suppressor genes or oncogenes (Table 2).[35,40,43,46,50,64,65]

MALIGNANT LYMPHOMAS

Malignant lymphomas consist of a diversity of different histology types. This wide heterogeneity of lym-

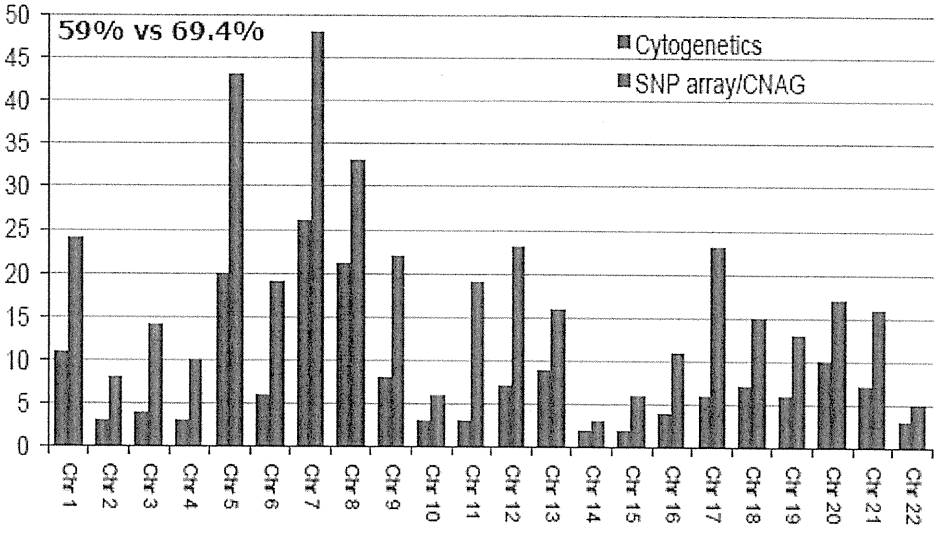

Figure 8. Numbers of genetic lesions in MDS and related myeloid neoplasms detected by SNP-A and metaphase karyotyping. The numbers of genetic lesions detected in a cohort of MDS, MDS/MPN, and sAML were compared between SNP-A (red bars) and metaphase karyotyping (blue bars) in each chromosome. The comparison was made among the 173 cases, in which successful metaphase karyotype data had been obtained.

Table 2. aUPDs and Their Gene Targets in Hematologic Neoplasms

Chromosome	Disease(s)	Gene Target(s)		References
1p13.1	MDS	Nras	Mutations	35,50
1p34	MPN, RARSt	cMPL	Mutations	49,64,72
4q24	MDS, MPN	TET2	Mutations	44
6q23	MALT, DLBCL	A20	Mutation or deletion	30
7q35	MDS, MDS/MPN	EZH2	Mutations	65
9p21	ALL	CDKN2A	Deletion	29
9p24	MPN	JAK2	Mutations	25,46
11p13	AML	WT1	Mutations	51
11q23.3	MDS/MPN	c-CBL	Mutations	35,50
13q12	AML	FLT3	ITD	51
13q14.3	CLL	miR-15a, miR-16-1	Deletion	69
17p13.1	AML, MDS	TP53	Mutations	35,73
17q11.2	JMML	NF1	Mutations	74
19q13.1	AML	CEBPA	Mutations	51
21q22.3	AML, MDS	RUNX1	Mutations	35,51

Abbreviations: MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; RARSt, refractory anemia with ring sideroblasts and thrombocytosis; MALT, mucosa-associated lymphoid tissue-derived lymphoma; DLBCL, diffuse large B-cell lymphoma; ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; JMML, juvenile myelomonocytic leukemia.

phomas has been confirmed by SNP-A karyotyping, in terms of the distribution of their genetic abnormalities, including copy number gains and losses, as well as aUPD, conferring unique genomic profiles to each lymphoma subtype.[30] For example, gains of both chromosomes 3 and 18, as well as focal deletions at the A20 locus, are a common feature of mucosa-associated lymphoid tissue (MALT)-derived lymphoma, while mantle cell lymphomas show recurrent deletions in the segments of 1p, 10p, and 11q, and gains of 3q, 8q, and 18q.[30] On the other hand, diffuse large B-cell lymphomas and follicular lymphoma show largely similar genomic profiles, including gains/amplifications involving the c-rel locus, and gains of 1q and chromosomes 3, 7, 12, and 18, indicating a common genetic background in both subtypes. aUPD is found in about 80% of follicular center-derived lymohomas and less frequently found in MALT and mantle cell lymphomas.[30,45,66] In follicular center-derived lymphomas, common targets of aUPD include 1p, 1q, 6p, 9p, 12q, and 17q, whereas 6qUPD is characteristic to MALT-type lymphoma.[30,45,66] Similar to aUPD in myeloid cancers, discrete gene targets have been clarified for some aUPDs in lymphomas, including HLA associated with 6pUPD, A20 with 6qUPD, and CDKN2 with 9pUPD, although the genetic targets of common aUPDs in 1p, 12q, and 17q have not been elucidated.[30]

CHRONIC LYMPHOCYTIC LEUKEMIA AND MULTIPLE MYELOMA

Chronic lymphocytic leukemia (CLL) and multiple myeloma (MM) are also among good indications for SNP-A karyotyping, because difficulty in obtaining metaphases frequently prevents successful conventional karyotyping. SNP-A analysis can sensitively detect genetic lesions in more than 80% of CLL cases, including frequent homozygous deletions involving the miR15a/miR16-1 locus, as well as gains of chromosome 12 associated with poor prognosis.[67-69] Other common genetic lesions in CLL detected by SNP-A karyotyping include recurrent deletions in 5q, 6q, 11q, and 17p, where the common deletion in 6q and 11q contains *AIM1* and *ATM,* respectively. Because of a high median age of CLL cases, aUPD should be carefully discriminated from IBD alleles. After excluding suspected IBD alleles, aUPD was relatively uncommon, being found in four of 56 cases, which involved 11q, 13q, and 17p.[68]

SNP-A karyotyping also can be applied to MM, but frequent low tumor contents in myeloma specimens may compromise the sensitivity of detecting genetic lesions. To keep the sensitivity of SNP-A karyotyping, enrichment of myeloma cells has been performed by sorting $CD138^+$ fractions.[70] As for the copy number changes, comparative results were obtained between array CGH and SNP-A karyotyping. Common genetic changes detected by SNP-A karyotyping include gains of 1q, 6p, and 11q and whole chromosomes 3, 5, 7, 9, 15, and 19, typically associated with hyperploidy, and deletions in 1p, 8p, and 16q and whole chromosomes 13 and X.[70,71] SNP-A karyotyping showed concordant results with those from FISH experiments in most cases, except for rare tetraploid samples, which were erroneously analyzed as diploid in SNP-A karyotyping.

aUPD is also common in MM with the median number of regions showing aUPD being three.[70]

CONCLUSION

SNP-A karyotyping represents one of the recent technological advances in the field of cancer genomics. It has enabled high-throughput analysis of genetic lesions in human cancers in terms of copy number alterations and allelic imbalances, unveiling a number of novel genetic targets and mechanisms that are involved in cancer development. Given such high performance of SNP-A karyotyping, it could be potentially applicable to bedside diagnosis and the clinical management of patients. While there exist accumulating observations that suggest diagnostic and/or prognostic values of SNP-A karyotyping, they need to be confirmed through more controlled studies. For example, when evaluating those abnormalities whose clinical values have been well established, SNP-A karyotyping would complement and even outperform metaphase karyotyping. On the other hand, SNP-A karyotyping will identify large numbers of novel genetic lesions whose clinical significance needs to be clarified before their clinical use, which might not always feasible with realistic numbers of cases due to higher heterogeneity these lesions could reveal. Clearly, more works should be required to establish the clinical values of SNP-A karyotyping technologies.

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