Identify latent chromosomal aberrations relevant to myelodysplastic syndromes

Qibin Song1, Yuxin Chu1, Yi Yao1, Min Peng1, Weihong Yang2, Xiaoqing Li2 & Shiang Huang2

Myelodysplastic syndromes (MDS) are a group of heterogeneous hematologic malignancies. This study aims to identify latent chromosomal abnormalities relevant to MDS, which may optimize the current diagnosis of MDS. Affymetrix CytoScan 750K microarray platform was utilized to perform a genome-wide detection of chromosomal aberrations in the bone marrow cells of the patients. The findings were compared with the results from traditional karyotypic analysis and FISH to reveal latent chromosomal aberrations. Chromosomal gain, loss, and UPD, and complex karyotypes were identified in those samples. In addition to established cytogenetic aberrations detected by karyotypic analysis, CytoScan 750K microarray also detected cryptic chromosomal lesions in MDS. Those latent defects underlying multiple gene mutations may construe the clinical variability of MDS. In Conclusion, Affymetrix CytoScan 750K microarray is efficient in identifying latent chromosomal aberrations in MDS.

Myelodysplastic syndromes (MDS) constitute a group of heterogeneous premalignant disorder of clonal hematopoietic stem cells (HSC), typically characterized by hypercellular bone marrow with immature blood cell lineages, leading to ineffective hemopoiesis, dysplasia, peripheral blood cytopenia, and frequent evolution to acute myeloid leukemia (AML)1. MDS often affects the elderly patients with a mean age of 70 years, with an incidence of 3-5/100000 persons2. Chromosomal abnormalities are frequently found in the bone marrow cells of about 50–60% primary MDS and in 80% of secondary MDS patients3. Since chromosomal lesions have a great influence on the diagnosis and prognosis of MDS, it has become clear that precise cytogenetic analysis is vital for an accurate diagnosis of MDS4. Common chromosomal aberrations include copy number variation (CNV), acquired uniparental disomy (UPD), and complex karyotypes. Chromosomal gain may engender the amplification of oncogenes. On the contrary, chromosomal loss may lead to deletion of tumor suppressor genes (TSGs)5. UPD results from mitotic recombination when segments of homologous chromosomes are exchanged, hence both copies of a chromosome pair that are inherited from one parent. Identification of UPD has important significance for investigating the pathogenesis of MDS6. Complex chromosomal aberrations (≥3 aberrations) are found in about 20% of MDS patients and are related to an increased risk of progress into AML with unfavorable prognosis7. More importantly, additional previously cryptic chromosomal lesions may affect the phenotypes of well-established aberrations. Those small cryptic aberrations may have diagnostic significance.

Currently, traditional metaphase cytogenetics (MC) still remains a gold standard in karyotype analysis of MDS. However, 40–50% of myelodysplastic syndromes (MDS) patients do not exhibit karyotypic abnormalities that can be detected by classical cytogenetic techniques8. Especially, UPD is not recognizable by MC because the chromosome banding patterns remain preserved9. Fluorescence in situ hybridization (FISH) may complement MC analysis, but its application is confined to identify particular chromosomal lesions by the probes utilized. The genetic complexity of malignant cells implores more precise genome-wide techniques, in order to identify some cryptic chromosomal aberrations in mixed cell lines10. The advent of high-resolution single nucleotide polymorphism array (SNP-A) technique has enabled a genome-wide scanning of specific chromosomal abnormalities previously undetectable by conventional MC or FISH10.

In this study, we have demonstrated the feasibility of applying Affymetrix CytoScan 750K Microarray to identify chromosomal CNV, UPD, and complex karyotypes in MDS patients. We postulate that Affymetrix CytoScan 750K Microarray would not only identify established chromosomal defects, but also reveal previously subliminal chromosomal lesions in MDS. Identification of those latent chromosomal aberrations may contribute to the stratification of subtypes in MDS, assign appropriate phenotypes, and design individualized treatment.

1Cancer Center, Renmin Hospital of Wuhan University, Wuhan, China. 2Molecular department, Kindstar global, Wuhan, China. Correspondence and requests for materials should be addressed to Q.S. (email: qibinsong@163.com)
Results

Clinical features of the patients. Conventional metaphase cytogenetic assay, FISH and Affymetrix Cytoscan 750 K Microarray were utilized to detect the common and latent chromosomal lesions for the patients. We selected 25 representative patients for our study, including 17 male and 8 female. Their ages range from 4-86 years old. 10 patients with CNV, 10 patients with UPD, and 5 patients with complex karyotypes were typically presented. The cohort comprises patients with RA (n = 4), RARS (n = 3), RCMD (n = 3), RCMD-RS (n = 1), RAEB-1 (n = 2), RAEB-2 (n = 2), 5q- syndrome (n = 1), MDS-U (n = 1), sAML (n = 8).

CNV. High resolution genome-wide Affymetrix Cytoscan 750 K Microarray is able to detect CNV larger than 100 Kb\textsuperscript{11}. As for case 1#, 3#, 4#, 5#, MC presented concordant results with microarray, yet microarray provided more precise chromosomal lesions in the samples. For instance, in case 1#, MC only revealed trisomy 8. By contrast, Cytoscan 750 K Microarray exhibited additional cryptic gain(3q27.1-qter) and loss(6q23.2-qter). Specific lesions can be seen in Table 1, Supplementary Figure S1 and Table S1. In case 2#, MC revealed discordant result: t(3;21)(q26;q22). Cytoscan 750 K Microarray didn't demonstrate this translocation, but unravelled cryptic gain(Yq11.222-pter) and loss(Yq11.222-qter) (detail in Supplementary Figure S2.1,2 and Table S2).

From case 6# to case 10#, MC presented normal karyotypes. By contrast, Cytoscan 750 K Microarray disclosed loss(20q11.23-q13.13) in case 6#, loss(13q13.1-q21.33) in case 7#, loss(14q11.2) and gain(1q21.1-q32.2) in case 8# (Fig. 1), loss(5q15-q22.3) in case 9#, and copy number state of these chromosomal lesions are available in our supplementary file. Those cryptic aberrations disclosed by Cytoscan 750 K Microarray are important for characterizing the patients with a diagnosis of MDS.

UPD. UPD still remains indiscernible by classical cytogenetic techniques, because it doesn't change chromosomal banding patterns\textsuperscript{12}. In this cohort, UPD in 10 MDS patients have not been detected by MC but by Cytoscan 750 K Microarray. For instance, in case 13#, although MC exhibits normal chromosome 11, Cytoscan 750 K Microarray disclosed loss(1q21.1-q32.2) with a large size.

Table 1. Comparison of CNV detected between Karyotypic analysis and Microarray.

| Patient NO. | Gender | Age (y) | Diagnosis       | MC                                      | Microarray                                      |
|-------------|--------|---------|-----------------|-----------------------------------------|------------------------------------------------|
| 1#          | female | 71      | sAML            | 47,XX,+8[20]                            | 3q27.1-qter +8                                   |
|             |        |         |                 |                                         | 6q23.2-qter                                    |
| 2#          | male   | 86      | sAML            | 46,XY,t(3;21)(q26;q22) [19]/46,XY[1]    | Yq11.1222-pter                                   |
|             |        |         |                 |                                         | Yq11.222-qter                                   |
| 3#          | male   | 69      | RAEB-2          | 46,XY,-20,+mar[16]/46,XY[4]            | 20q13.2qter                                     |
|             |        |         |                 |                                         | 20p11.1-pter; 20q11.21-q13.2                    |
| 4#          | male   | 81      | RCMD            | 46,X,-Y,+8[12]/46,XY[8]                | +8                                              |
|             |        |         |                 |                                         | Yp11.31-q11.23                                  |
| 5#          | female | 60      | RCMD            | 46,XX dél(20)[q11][20]                | 1q21.2; 20p11.1                                  |
|             |        |         |                 |                                         | 20q11.23-q13.32                                 |
| 6#          | male   | 85      | RAEB-1          | 46,XY                                  |                                                  |
|             |        |         |                 |                                         |                                                  |
| 7#          | female | 71      | RAEB-1          | 46,XX                                  |                                                  |
|             |        |         |                 |                                         |                                                  |
| 8#          | female | 51      | RA              | 46,XX                                  | 1q21.1-q32.2                                    |
|             |        |         |                 |                                         | 1q11.2                                           |
| 9#          | male   | 31      | 5q- syndrome    | 46,XY                                  |                                                  |
|             |        |         |                 |                                         |                                                  |
| 10#         | male   | 78      | RARS            | 46,XY                                  |                                                  |

Table 1. Comparison of CNV detected between Karyotypic analysis and Microarray.

Figure 1. Comparison of chromosomal gain detected between MC and microarray. Although MC indicates normal chromosomal 1 in case 8#, Affymetrix Cytoscan 750 K Microarray still reveals cryptic gain(1q21.1-q32.2) with a large size.
Complex chromosomal lesions. Complex karyotypes in MDS often encompass three or more chromosomal abnormalities and are associated with unfavorable clinical outcomes. Karyotypic analysis and Cytoscan 750 K Microarray have illustrated complex chromosomal lesions in 5 cases in point. Despite the fact that many large genomic aberrations were detected by metaphase cytogenetic assay, more hidden chromosomal loss and gain, especially UPD were still revealed by Cytoscan 750 K Microarray. In case 21#, MC indicates 46,XY, ins(1)(p13p22p36), add(2)(q31), −5, −3, −7, add(8)(p21), +9, add(9)(q34), −11, +mar1, +mar2, inc[cp6]. Comparatively, Cytoscan 750 K Microarray has revealed loss(1p22.3p21.2, 1p36.13p36.11, 5q14.3q21.3, 7q21.3q36.3, 12p13.31p12.1, 18q12.3qter), gain(8q11.1q24.3, 11p12q22.1, 13q11-q12.3), and UPD(7q22.1q31.2, 17p13.3p11.2) (Table 3, Supplementary Figure S21). As for case 22#, in spite of some “del” and “add” determinied by karyotypic analysis, Cytoscan 750 K Microarray still disclosed a complex pattern of gains, losses and UPD in this case (Table 3, Supplementary Figure S22). Case 23#, 24#, and 25# have a series of complex chromosomal aberrations identified by Cytoscan 750 K Microarray that complement the results revealed by MC. By contrast, microarray analysis revealed cryptic UPD and a lot of smaller lesions, such as UPD(3p21.31-p21.1) and loss(3p14.1-q21.1, 5q13.3-q35.1, 9q21.11-q31.1) in case 25# (Table 3, Supplementary Figure S25). Generally, Cytoscan 750 K Microarray revealed complex rearrangements with multiple gains and losses. But MC indicated undefined materials exhibited as marker chromosomes (“+mar”) and chromosomal additions (“add”) in some cases. Detailed lesions of complex karyotypes have been listed in Table 3 and our Supplementary file.
Validation of microarray results. The resolution of conventional metaphase cytogenetic analysis is approximately 5 Mb\(^ {14}\). In order to validate those lesions identified by microarray, we also initiated FISH to detect the chromosomal aberrations in some of those cases. For instance, in case 17#, UPD(3p21.31p21.1), UPD(9p21.1pter), and UPD(11p11.2p11.12) haven’t been detected by MC but by microarray. FISH also exhibit normal chromosome 5, 7, 8, 20 (Supplementary Figure S17, Table S17). In contrast, MC revealed +5 and del(5)(q13q31) in case 24#, while Cytoscan 750 K Microarray disclosed gain(5q14.2pter) and loss(5q14.3qter). FISH indicated loss of CSF1R signal, representing loss(5q33-34). So genomic loss in chromosomal 5 has been confirmed by FISH (Fig. 4). In addition, trisomy 8 in case 24# has also been validated by FISH (Supplementary Figure S24).

Discussion

Chromosomal abnormalities are frequent in MDS and have many clinical implications. However, a great amount of patients don’t indicate cytogenetic abnormalities. MDS patients with the same chromosome lesions may have heterogeneous phenotypes, implying that some hidden genomic changes may exist among the patients\(^ {15}\). In the present study, we utilized traditional MC and Affymetrix Cytoscan 750 K Microarray to identify latent chromosomal changes in a cohort of 25 patients. Our results indicated that high-resolution Affymetrix Cytoscan 750 K Microarray improves the identification of chromosomal aberrations by karyotypic analysis in MDS.
Gain and loss of gene copies may result in gene over-expression, absence of any functional transcript, or modest changes in gene expression. We have unveiled chromosomal aberrations in regions defined as CNV of contiguous clones undetectable by classical karyotypic analysis. For instance, we identified cryptic gain (3p27.1-qter) and loss (6q23.2-qter) in case 1#. In this region, Loss(6q23.2-q23.3) has been reported to involve in sAML. Furthermore, cryptic chromosomal aberrations detected by microarray have been useful for a comprehensive analysis of gene mutations in MDS. The copy number status of TET2 (on 4q24), IRF1 (5q31.1), NPM1 (5q35.1), LMAB4 (7q31.1), EZH2 (7q36.1), ETV6 (12p13.2), TP53 (17p13.1), NFI (17q11.2), ASXL1 (20q11.21), RUNX1 (21q22.12), and STAG2 (Xq25) have been investigated in a large cohort of MDS patients. In our cohort, we have identified a 1.48 Mb loss at 4q24 in case 10#. TET2 gene in this region is a tumor-suppressor gene. The deletion or mutation of TET2 often predict inferior prognosis in patients with chronic myelomonocytic leukemia. Although aberration in chromosome 5 has been considered as the most prevalent chromosomal lesion in MDS, some cryptic defects which affect additional key genes have not been clarified comprehensively. The major commonly deleted region (CDR) has been delineated at band 5q31.1. We have identified a 97.71 Mb loss(5q14.3qter) in case 24#. Deletion of 5q may engender haploinsufficiency of many critical genes, including ribosomal protein S14 (RPS14), cassein kinase 1 αI (CSNK1A1), adenomatous polyposis coli (APC), heat shock protein family A (HSP70) member 9 (HSPA9), early growth response 1 (ERG1), DEAD-box helicase 41 (DDX41), NPM1, TRAF-interacting protein with forkhead- associated domain B (TIFAB), Diaphanos-related formin 1 (DIAPH1), microRNA (miR)-145 and miR-146a. Haploinsufficiency for the ribosomal gene RPS14 has been reported to impede erythroid differentiation in the 5q- syndrome. Haploinsufficiency deletion of CSNK1A1 may upregulate WNT signaling and stimulate stem cell expansion. Loss(5q14.3qter) in case 24# has been validated by FISH. We have also detected loss(7q11.21) in case 10# and monosomy 7 in case 22#, 23#. Deletion of 7q and monosomy 7 are also prevalent in MDS and often portend an unfavorable outcome. These chromosomal alterations can also cause haploinsufficiency of some key genes implicated in MDS. These genes comprise EZH2, CUX1 and MLL3. MLL3 haploinsufficiency cooperates with RAS mutation and Trp53 to exacerbate leukemia. In addition, we have found gain(20q13.2qter) in case 3#. MacKinnon et al. have investigated AML and MDS patients with 20q amplification. They identified a 250 kb common region which subsumed HCK, TM9SF4, PLAGL2, and POFUT1 gene. These patients often had a higher proportion of erythroblasts. The amplification of 20q portends the existence of oncogene. Generally, these latent chromosomal aberrations contain a lot of key genes which are tightly associated with the pathogenesis of MDS. CNV identified by Affymetrix Cytsocan 750 K Microarray can contribute to the differential diagnosis of subtypes in MDS.

Recent investigations have indicated that UPD can be responsible for homozygosity of mutations of critical genes within chromosomal regions. Reduction to homozygosity as a result of UPD was preliminarily considered to be a mechanism for the inactivation of tumor suppressor genes. We have demonstrated UPD in 10 cases with normal karyotypes. For example, we detected UPD(13q11-qter) in case 12#. FLT3 gene in 13q12 encodes class III receptor tyrosine kinase that regulates hematopoiesis. FLT3-ITD internal tandem duplications have been observed during disease progression and confers an unfavorable prognosis. We also identified UPD(11p11.2-ppter) in case 13#. WT1 gene mutation has been reported in UPD 11p which is related to the pathogenesis of AML. Furthermore, we have also found UPD(11q13.1-qter) in case 15#. The c-CBL gene is located in 11q23.3. Clonal selection of UPD 11q and CBL gene mutation often reflected the progression of MDS to AML. It is noteworthy that in RCMD case 16#, the UPD of region 17q22-qter harbored the ETV4 gene, which encodes an ETS transcription factor indispensable for hematopoiesis. Additionally, we have found UPD(9p21.1-ppter) in case 17#. UPD 9p is tightly associated with a homozygous activating JAK2(V617F) gene mutation, implying serious prognosis. Consequently, nonrandom segmental UPDs identified in this cohort may contribute to the investigation of the pathogenesis of MDS underlying large deletions.

MDS with complex chromosomal aberrations often herald short survival and an increased risk of evolution to AML. Complex karyotypes with multiple chromosomal changes are found in about 20% of newly diagnosed MDS patients and are relevant to a poor prognosis. We exhibited 5 cases with complex chromosomal lesions, which have been listed in our supplementary file. A combination of traditional karyotypic analysis with Affymetrix Cytsocan 750 K Microarray may well provide a more comprehensive detection of complex chromosomal aberrations in MDS.

Given the recent discovery of many recurrent gene mutations in MDS, it's still urgent to validate prior mutational correlative data. The temporal order of mutation acquisition has reflected the importance of subclonal genetic events in MDS. For instance, mutations impacting RNA splicing and DNA methylation occur early in disease progression, while kinase activating mutations (such as KIT and NRAS) occur even later in disease progression. Early detection of subclonal mutations may reflect significant prognostic variables in MDS. In our cohort, we have found many discrepancies between MC and Cytsocan 750 K Microarray. Microarray analysis has a variable ability to detect mosaicism that FISH and karyotyping may not accurately detect the level of mosaicism. The most likely reason for these discrepancies is probably that some aberrations are subclonal.

On the other hand, one criticism of microarray for detecting chromosomal aberrations in MDS is the possibility of "false positive" results or findings of unclear clinical significance. A proportion of alterations identified in the patients may reflect normal age-related chromosomal changes. For instance, we have found loss(Yq11.222-qter) in case 2#. Loss of the Y-chromosome (LOY) is described as both a normal age-related event and a marker of a neoplastic clone in hematologic diseases. Paired normal DNA from the same MDS patient may reduce the number of false positives generated by microarray.

Additionally, some patients with clonal cytopenia of undetermined significance (CCUS) that do not meet the criteria for MDS may also benefit from SNP-A. A recent study has applied combined comparative genomic hybridization and SNP-A to detect cryptic chromosomal lesions in both MDS and cytopenias of undetermined significance. Based on the combined array findings, 42% of patients with indeterminate morphologic findings were categorized as CCUS. Cryptic array findings among those patients comprised large-scale UPD (up to
118 Mb) and genomic deletion of loci implicated in MDS pathogenesis (eg, TET2 (4q22) and NUP98 (11p15)). The latent chromosomal lesions revealed by SNP-A helped to indicate clonal hematopoiesis and prompted classification as CCUS. Hence microarray analysis significantly improves the detection rate of clinically significant findings.

In conclusion, Affymetrix Cytoscan 750 K Microarray have identified many cryptic chromosomal abnormalities relevant to MDS, which may interpret the clinical variability and enhance our understanding of the pathogenesis of MDS.

**Material and Methods**

**Patients and Specimen.** The cohort of this study comprises patients whose bone marrow aspirates were recruited in Kingstar Global company for pathologic diagnosis of MDS from December 2014 to July 2015. All specimens were acquired with patients' approval, under the protocols permitted by Institutional Ethics Committee of Wuhan university, in comply with Helsinki Declaration. Informed consent was signed for each patient. And any publication of identifying information was also approved by the participants.

**Cytogenetic analysis.** Traditional G-banding Karyotypic analysis was initiated on bone marrow aspirates by trypsin and Giemsa dye. Short-term cell cultures were carried out in medium supplemented with GM-CSF or conditioned medium III. Then the cells were harvested and metaphase preparations were performed according to standard procedures. Karyotypes were depicted in the light of International System for Human Cytogenetic Nomenclature 2016. At least 20 metaphases per sample should be analyzed whenever possible.

**FISH Fluorescence in situ hybridization** (FISH) was performed according to the manufacturer's protocols, in order to validate chromosomal aberrations detected by Affymetrix Cytoscan 750 K Microarray. A total of 400 interphase nuclei were evaluated by two independent pathologists under fluorescent microscope. The locus-specific probes were displayed in Table 4.

**DNA preparation.** DNA was extracted from bone marrow of individual patients using the QIAamp DNA Blood Mini Kit according to the manufacturer's instructions. The concentration and quality of DNA samples were evaluated by Nanodrop 2000 spectrophotometer (Thermo Scientific). DNA integrity was assessed by 1% agarose gel electrophoresis. The quality controls (QC) of Affymetrix CytoScan 750 K microarray required that DNA concentration should be no less than 50 ng/µL, OD260/280 ≈ 1.9, OD260/230 ≈ 2.0.

**Cytoscan 750 K Microarray Assay.** Affymetrix Cytoscan 750 K Microarray provides a genome-wide coverage on cytogenetic relevant regions, including 550,000 markers for detecting copy number variation and 200,000 high performing SNP probes with genotype accuracy > 99%. All probes are empirically selected for exceptional performance. The Affymetrix® CytoScan™ Assay protocol is optimized for processing 8 to 24 samples at a time to obtain whole genome copy number and SNP information. The Workflow of CytoScanTM Assay can be briefed as follows (Fig. 5):

1. Digestion of gDNA with Nsp I restriction endonuclease.
2. Ligation with Adaptor and T4 DNA Ligase.
3. Polymerase chain reaction (PCR) to amplify Ligated Samples and PCR Product Check.

### Table 4. The probes and targets of FISH.

| Probe          | Target                  |
|---------------|-------------------------|
| D5S23/D5S721/CSF1R | 5p15.2/5q33-34          |
| D7Z1/D7S486   | 7p11.1-7q11.1           |
| D6Z2          | 8p11.1-q11.1            |
| D20S108       | 20q12                   |

![Figure 5. The Workflow of Affymetrix Cytoscan 750 K Microarray. QC1 defines DNA concentration ≥ 50ng/µL, OD260/280 ≥ 1.9, OD260/230 ≥ 2.0. QC2 defines PCR products on 1% gel electrophoresis should be 150 bp-2000 bp, Purified PCR products ≥ 300 ng/µL, OD260/280 ≥ 1.9, OD260/230 ≥ 2.0. QC3 defines fragmentation products on 1% gel electrophoresis should be 25 bp-125 bp. QC4 defines: SNPQC ≥ 15.0; MAPD ≤ 0.25; Waviness SD ≤ 0.12.](image-url)
(4) PCR Product Purification with magnetic beads.
(5) Quantitation of purified samples.
(6) Fragmentation of Purified PCR Products and QC Gel Analysis.
(7) Labeling the Fragmented DNA with TdT enzyme.
(8) Hybridization with CytoScan 750 K Microarray at 50 °C oven for 16 to 18 hours.
(9) Wash and Stain the genechips on Fluidics Station.
(10) Scan the arrays in optic GeneChip Scanner 3000.

Data analysis. The data of Cytoscan 750 K Microarray were analyzed using Chromosome Analysis Suite Version 2.0 (Affymetrix). The QC thresholds were: SNPQCC ≥ 15.0; MAPD ≤ 0.25; Waviness SD < 0.12. These QC metrics can evaluate the overall quality of SNP array data. Median Absolute Pairwise Difference (MAPD) represents the typical distance between marker pairs with respect to log2 ratios. SNPQCC measures the degree of separation between genotype clusters aggregated across multiple markers. Waviness-SD gauges the differences between probe sets. The microarray data were interpreted according to the annotations of genome version GRCh37 (hg19). Only the samples which complied with QC criteria and identified CNV with over 100 Kb and at least 10 aberrant probes were chosen for further analysis. Identified CNVs were contrasted with the Database of Genomic Variants (http://dgv.tcag.ca/dgv/app/home) to exclude the polymorphic variations in healthy population. As for UPD, we used an algorithm that regards both location and size of >5 Mb aberrations in order to preclude nonclonal regions. To reckon the size of the affected genome in each patient, we recognized the total size of alterations in chromosomes, including CNV and UPD.

References
1. Lukackova, R., Gerykova, Bujalkova, M., Majerova, L. & mladosievicova, B. Molecular genetic methods in the diagnosis of myelodysplastic syndromes. A review. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 158, 339–345 (2014).
2. Schlegelberger, B. et al. Update on cytogenetic and molecular changes in myelodysplastic syndromes. Leuk Lymphoma 53, 525–536 (2012).
3. Zemanova, Z. et al. Involvement of deleted chromosome 5 in complex chromosomal aberrations in newly diagnosed myelodysplastic syndromes (MDS) is correlated with extremely adverse prognosis. Leuk Res 38, 537–544 (2014).
4. Platbecker, U. et al. Update on developments in the diagnosis and prognostic evaluation of patients with myelodysplastic syndromes (MDS)-consensus statements and report from an expert workshop. Leuk Res 36, 264–270 (2012).
5. Mitelman, F., Johansson, B. & Mertens, F. Mitelman database of chromosome aberrations and gene fusions in cancer. Available from: http://cgap.nci.nih.gov/Chromosomes/Mitelman (2016).
6. Gondekk, L. P. et al. Single nucleotide polymorphism arrays complement metaphase cytogenetics in detection of new chromosomal lesions in MDS. Leukemia 21, 2058–2061 (2007).
7. Svobodova, K. et al. Copy number neutral loss of heterozygosity at 17p and homozygous mutations of TP53 are associated with complex chromosomal aberrations in patients newly diagnosed with myelodysplastic syndromes. Leuk Res 42, 7–12 (2016).
8. Thiel, A. et al. Comprehensive array CGH of normal karyotype myelodysplastic syndromes reveals hidden recurrent and individual genomic copy number alterations with prognostic relevance. Leukemia 25, 387–399 (2011).
9. Simons, A. et al. Genome-wide arrays in routine diagnostics of hematological malignancies. Hum Mutat 33, 941–948 (2012).
10. Huh, J. et al. Different characteristics identified by single nucleotide polymorphism array analysis in leukemia suggest the need for different application strategies depending on disease category. Genes Chromosomes Cancer 52, 4455 (2013).
11. Shin, S. et al. Routine Chromosomal Microarray Analysis is Necessary in Korean Patients With Unexplained Developmental Delay/Mental Retardation/Autism Spectrum Disorder. Ann Lab Med 35, 510–518 (2015).
12. Hu, Q. et al. The prevalence of chromosomal aberrations associated with myelodysplastic syndromes in China. Ann Hematol 95, 1241–1248 (2016).
13. Kolquist, K. A. et al. Microarray-based comparative genomic hybridization of cancer targets reveals novel, recurrent genetic aberrations in the myelodysplastic syndromes. Cancer Genet 204, 603–628 (2011).
14. Iafrate, A. J. et al. Detection of large-scale variation in the human genome. Nat Genet 36, 949–951 (2004).
15. Gondekk, L. P. et al. Detection of cryptic chromosomal lesions including acquired segmental uniparental disomy in advanced and low-risk myelodysplastic syndromes. Exp Hematol 35, 1728–1738 (2007).
16. Mayrhofer, M., Viklund, B. & Isaksson, A. Rawcopy: Improved copy number analysis with Affymetrix arrays. Sci Rep 6, 36158 (2016).
17. Gondekk, L. P. et al. Chromosomal lesions and uniparental disomy detected by SNP arrays in MDS, MDS/MPD, and MDS-derived AML. Blood 111, 1534–1542 (2008).
18. Haferlach, T. et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. Leukemia 28, 241–247 (2014).
19. Ganguly, B. B. & Kadam, N. N. Mutations of myelodysplastic syndromes (MDS): An update. Mutat Res Rev Mutat Res 769, 47–62 (2016).
20. Cui, Y. et al. TET2 mutations were predictive of inferior prognosis in the presence of ASXL1 mutations in patients with chronic myelomonocytic leukemia. Stem Cell Invest 3, 50 (2016).
21. Spellicer, A. S., Gibson, C. J. & Ebert, B. L. The genetics of myelodysplastic syndrome: from clonal hematopoiesis to secondary leukaemia. Nat Rev Cancer 17, 5–19 (2017).
22. Schneider, R. K. et al. Rps11 haploinsufficiency causes a block in erythroid differentiation mediated by S100A8 and S100A9. Nat Med 22, 288–297 (2016).
23. Schneider, R. K. et al. Role of casein kinase 1A1 in the biology and targeted therapy of del(5q) MDS. Cancer Cell 26, 890–920 (2014).
24. Krones, J. et al. Lenalidomide induces ubiquitination and degradation of CK1ε in del(5q) MDS. Nature 523, 183–188 (2015).
25. Hasle, H. Myelodysplastic and myeloproliferative disorders of childhood. Hematology Am Soc Hematol Educ Program 2016, 598–604 (2016).
26. Chen, C. et al. MLL3 is a haploinsufficient 7q tumor suppressor in acute myeloid leukemia. Cancer Cell 25, 652–665 (2014).
27. McNemey, M. E. et al. CUX1 is a haploinsufficient tumor suppressor gene on chromosome 7 frequently inactivated in acute myeloid leukemia. Blood 121, 973–983 (2013).
28. Nikoloski, G. et al. Somatic mutations of the histone methyltransferase gene EZH2 in myelodysplastic syndromes. Nat Genet 42, 665–667 (2010).
29. Mackinnon, R. N. et al. The paradox of 20q11.21 amplification in a subset of cases of myeloid malignancy with chromosome 20 deletion. Genes Chromosomes Cancer 49, 998e1013 (2010).
30. Hemmat, M. et al. Submicroscopic deletion of 5q involving tumor suppressor genes (CTNNA1, HSPA9) and copy neutral loss of heterozygosity associated with TET2 and EZH2 mutations in a case of MDS with normal chromosome and FISH results. *Mol Cytogenet* 7, 35 (2014).

31. Pellagatti, A. & Boulton, J. The molecular pathogenesis of the myelodysplastic syndromes. *Eur J Haematol* 95, 3–15 (2015).

32. Tsitsipatis, D. et al. Synergistic killing of FLT3ITD-positive AML cells by combined inhibition of tyrosine-kinase activity and N-glycosylation. *Oncotarget* 8, 26613–26624 (2017).

33. O’Keefe, C. et al. Copy neutral loss of heterozygosity: a novel chromosomal lesion in myeloid malignancies. *Blood* 115, 2731–2739 (2010).

34. Barresi, V. et al. Clonal selection of 11q CN-LOH and CBL gene mutation in a serially studied patient during MDS progression to AML. *Leuk Res* 34, 1539–1542 (2010).

35. Merkerova, M. D. et al. From cryptic chromosomal lesions to pathologically relevant genes: integration of SNP array with gene expression profiling in myelodysplastic syndrome with normal karyotype. *Genes Chromosomes Cancer* 51, 419–428 (2012).

36. Wafa., A. et al. A new complex karyotype in a unique de novo myelodysplastic syndrome case involving ten chromosomes and monoallelic loss of TP53. *Gene Reports* 4, 208–212 (2016).

37. Murphy, D. M. et al. NRAS mutations with low allele burden have independent prognostic significance for patients with lower risk myelodysplastic syndromes. *Leukemia* 27, 2077–2081 (2013).

38. Papaemmanuil, E. et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. *Blood* 122, 3616–3627 (2013).

39. Ganster, C. et al. New data shed light on Y-loss-related pathogenesis in myelodysplastic syndromes. *Genes Chromosomes Cancer* 54, 717–724 (2015).

40. Heinrichs, S. et al. Accurate detection of uniparental disomy and microdeletions by SNP array analysis in myelodysplastic syndromes with normal cytogenetics. *Leukemia* 23, 1605–1613 (2009).

41. Evans, A. G., Ahmad, A., Burack, W. R. & Iqbal, M. A. Combined comparative genomic hybridization and single-nucleotide polymorphism array detects cryptic chromosomal lesions in both myelodysplastic syndromes and cytopenias of undetermined significance. *Mod Pathol* 29, 1183–1199 (2016).

42. Stevens-Kroef, M., Simons, A., Rack, K. & Hastings, R. J. Cytogenetic Nomenclature and Reporting. *Methods Mol Biol* 1541, 303–309 (2017).

Acknowledgements
This study was supported by National Natural Science Foundation of China (NO. 81372407) and (NO. 81670123).

Author Contributions
Qibin Song, Yi Yao, Min Peng designed this study; Yuxin Chu wrote this manuscript; Weihong Yang and Xiaoqing Li performed experiments and analyzed the data; Shiang Huang managed patients and collected clinical samples.

Additional Information
Supplementary information accompanies this paper at doi:10.1038/s41598-017-10551-3

Competing Interests: The authors declare that they have no competing interests.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2017