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ORIGINAL ARTICLE

Retrospective evaluation of bone marrow cell morphology in a cohort of patients with isolated idic(20q-) karyotypic abnormalities

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Abstract

Isochromosome 20q- (i(20q-)), as a rare reproducible chromosomal anomaly formed on the basis of 20q-, has not been commonly reported. Due to the rarity of this karyotypic anomaly, the bone marrow morphological characteristics of the patients with i(20q-) have not been clarified until now. In this study, the bone marrow cell morphology from MDS patients with isolated i(20q-), isolated 20q-, and normal karyotype was retrospectively compared and statistically analyzed. The results indicated that the isolated i(20q-) was mostly detected in MDS-MLD patients. The frequency and proportion dysplasia of cytoplasmic vacuolization in erythoid cells and small or unusually large size in myeloid cells of isolated i(20q-) MDS patients were significantly higher than those of normal karyotype MDS patients respectively ($P < 0.05$); the frequency and proportion dysplasia of decreased granules/agranularity in myeloid cells of isolated i(20q-) MDS patients were higher than those of isolated 20q- MDS patients ($P < 0.05$). The incidence of some specific morphological manifestations, such as deeply lobulated and hyperlobulated megakaryocytes and hypogranular and vacuolized eosinophils, may be an important morphological implication for the anomaly of isolated i(20q-). These morphological features of dysplasia may be helpful in distinguishing MDS with isolated i(20q-) from those with isolated 20q- and normal karyotype.

Keywords Isolated i(20q-) · Isolated 20q- · Myelodysplastic syndrome · Morphological dysplasia

Introduction

The long arm deletion of chromosome 20 (20q-) is one of the most common karyotypic anomalies observed in the myeloid category of hematological diseases [1], and was ever reported in approximately 10% of myeloproliferative neoplasms (MPNs), 4% of myelodysplastic syndrome (MDS), and 1% of acute myeloid leukemia (AML) other than lymphocytic conditions [2]. Isochromosome 20q- (i(20q-)), a rare reproducible chromosomal anomaly formed on the basis of 20q-, was initially reported by Li et al. in 2004 [3] and was accumulatively reported in less than 60 patients so far. Due to the rarity of this karyotypic anomaly, the clinical and laboratorial characteristic of i(20q-) especially isolated i(20q-) is not clarified yet [4]. In the present study, we found that the isolated i(20q-) may be one of the recurrent abnormalities in MDS patients. Therefore, the bone marrow cell morphological features from MDS patients with isolated i(20q-), isolated 20q-, and normal karyotypes were retrospectively analyzed, in order to evaluate the potentiality of an unique bone marrow cell morphology in patients with isolated i(20q-) for clinical usage.

Patients and methods

Patients

A total of 11 MDS patients who were identified as isolated i(20q-) in the first affiliated hospital of Suzhou University between July 2002 and December 2017 as inpatients or outpatients with archived bone marrow smears were included in the present study. The subtypes of MDS included MLD ($n = 9$) and RS-MLD ($n = 2$). In addition, MDS patients with isolated 20q- ($n = 21$) and normal karyotype ($n = 24$) who had archived bone marrow smears were included as the comparators. The patients were categorized in accordance with the WHO Classification

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Criteria 2017 [5]. The demographic and other key characteristics of the three groups were summarized in Table 1.

Methods

Bone marrow cell cytogenetic analysis and gene mutation detection

Cytogenetic analysis was performed on bone marrow (BM) cells using direct method and/or 24-h culturing. An R-banding assay was used for karyotypic analysis. Clonal karyotype abnormalities were described according to the International System for Human Cytogenetic Nomenclature (ISCN, 2013) [6]. The confirmatory analysis was performed using dual-color fluorescence in situ hybridization (D-FISH) for patients with i(20q-) or 20q- who had archived chromosome suspension. The normal karyotype specimen from male person was used as the normal control. Of these MDS patients, 22 were detected using the gene mutation test, and the results were analyzed.

Bone marrow cell morphology analysis

The bone marrow smears of the patients were carefully observed by a senior hemo-morphologist and double checked by at least another senior morphologist before getting the final diagnosis.

The features of cellular morphological dysplasia were evaluated from the following three aspects: firstly, the "incidence of morphological dysplasia" (IMD) was evaluated in the patients respectively according to dysplasia type of erythroid, myeloid, and megakaryocytic lineages by 2017 WHO classification (IMD-WHO); secondly, "percentage of dysplastic cells" (PDC) respectively in erythroid, myeloid, and megakaryocytic lineage from marrow cells of each patient based on 2017 WHO classification (PDC-WHO) was calculated [7–9]; finally, the "IMD" and the "PDC" of specific morphological dysplasias described in the previous literature of i(20q-) patients were analyzed.

The "incidence of morphological dysplasia" (IMD) was calculated as the number of cases with the emerged morphological dysplasia divided by the number of total cases in each group (present as the percentage). The "percentage of dysplastic cells" (PDC) was determined as follows: for the erythroid and myeloid lineage, 100 cells from each lineage were screened for the presence of the morphological dysplastic features; for megakaryopoiesis, the number of dysplastic megakaryocytes as well as the total number of megakaryocytes observed was documented for the percentage of dysplastic megakaryocytes (that is, the number of dysplasia cells divided by 100 cells in each linage; present as the mean percentage). The types of morphological dysplasia for "incidence of morphological dysplasia" (IMD-WHO) and "percentage of dysplastic cells" (PDC-WHO) were defined according to the 2017 WHO classification (Fig. 1).

The "IMD" of specific morphological dysplasia reported in the previous literature of the i(20q-) patients was calculated as the number of cases demonstrating specific type of morphological dysplasia divided by number of total cases in each group (present as the percentage). The "PDC" of specific morphological dysplasia also according to the specific type from the previous literature was analyzed (present as the mean percentage). The specific morphological dysplasia previously described in i(20q-) patients included the type of hypogranulated and vacuolized neutrophils, hypogranular and vacuolized eosinophils, neutrophil erythrophagocytosis, and deeply lobulated and hyperlobulated megakaryocytes (Fig. 2) [10].

Statistical analysis

All the statistical procedures were performed using SPSS 18.0 software. The categorical data were analyzed using chi-square test or Kruskal–Wallis–H test. P value < 0.05 was considered to be statistically significant.

Results

Clinical data

From July 2002 to December 2017, among the inpatients and outpatients in our center who underwent routine karyotypic analysis, a total of 52 patients with i(20q-) were identified, of whom 32 had archived bone marrow smears. Of these patients, 13 patients were isolated i(20q-), and the bone marrow cell R-banded karyotype and FISH analysis of two representative patients were plotted in Fig. 3. Eleven of these 13 isolated i(20q-) patients were identified as MDS (85%). The

Table 1 The demographic characteristics of the MDS patients with isolated i(20q-), isolated 20q-, and normal karyotype

Patient group	Patient selection mode	*N*	M/F	Median age (years)
Isolated i(20q-)	Consecutive	11	0.83:1	70.0 (45–81)
Isolated 20q-	Consecutive	21	1.1:1	64.0 (20–87)
Normal karyotype	Random	24	2:1	44.0 (24–68)

N number of patients, *M* male, *F* female

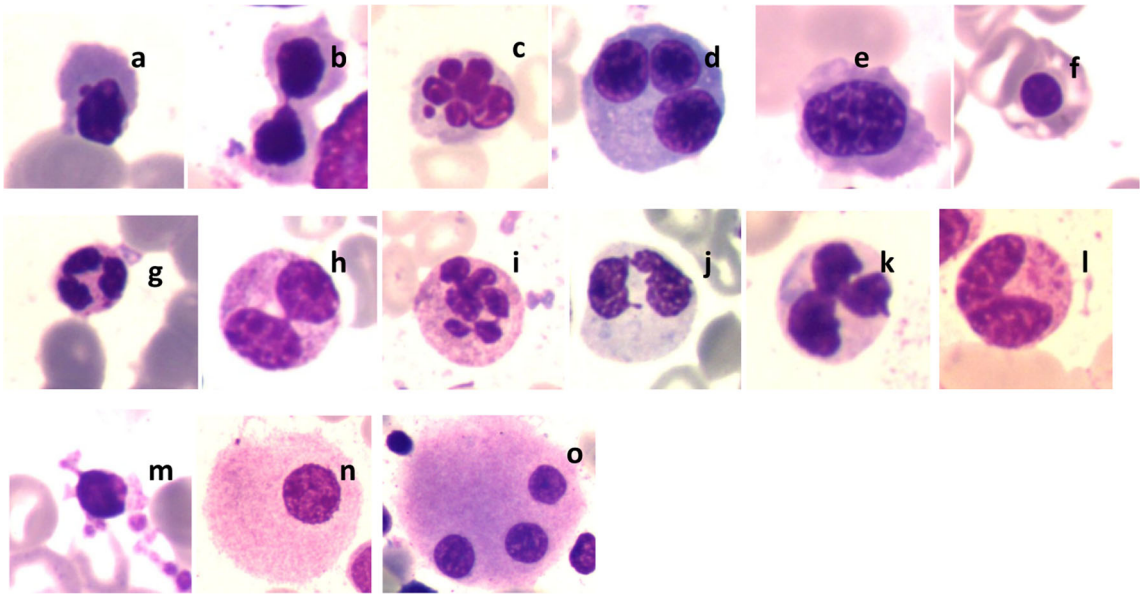

Fig. 1 Morphological dysplasias defined by 2017 WHO classification in erythoid lineage (**a**–**f**), myelocytic lineage (**g**–**l**), and megakaryocytic lineage (**m**–**o**). **a** Nucleus budding. **b** Internuclear bridging. **c** Karyorrhexis. **d** Multi-nuclearity. **e** Megaloblastic changes. **f** Cytoplasmic vacuolization (6 types in erythoid lineage). **g** Small or unusually large size. **h** Nuclear hyposegmentation. **i** Nuclear hypersegmentation. **j** Decreased granules/agranularity. **k** Dohle bodies. **l** Pseudo Chédiak–Higashi granules (6 types in myelocytic lineage). **m** Micromegakaryocyte. **n** Nuclear hypolobation. **o** Multinucleation (3 types in megakaryocytic lineage)

median age of the patients was 70 (45–81) years old and the male was less than the female (0.83:1).

Of the 97 patients who were identified having isolated 20q- with archived bone marrow smears from 2002 and 2017, 22 were diagnosed as MDS (22%), which were selected as the comparators. Additionally, 24 MDS patients with normal karyotype were randomly selected as the control group.

Analysis of IMD-WHO in erythroid, myeloid, and megakaryocytic lineages in MDS patients with isolated i(20q-), isolated 20q-, and normal karyotypes

As shown in Fig. 4a, for patients with isolated i(20q-), isolated 20q-, and normal karyotypes, the most frequently observed dysplasia in erythroid lineage was megaloblastic changes (82%, 100%, 67% respectively), which was almost found in more than 2/3 MDS patients; the next commonly observed was cytoplasmic vacuolization (73%, 57%, 33% respectively) in the three groups. The dysplasia of cytoplasmic vacuolization in erythroid lineage was observed at significantly higher frequency in patients with isolated i(20q-) than in patients with normal karyotype ($P < 0.05$). The dysplasias of nucleus budding and karyorrhexis in erythroid lineage in patients of isolated i(20q-) were numerically more common than those of the other two MDS groups without significant difference ($P > 0.05$).

As shown in Fig. 4b, for patients with isolated i(20q-), isolated 20q-, and normal karyotypes, the most frequently occurred dysplasia in myeloid lineage was small or unusually large size (91%, 95%, 50% respectively). Moreover, this dysplasia was more frequently found in patients with isolated i(20q-) and isolated 20q- than in patients with normal karyotype ($P < 0.05$). The incidences of nuclear hyposegmentation and decreased granules/agranularity in patients with isolated i(20q-) were higher than those in patients with isolated 20q- ($P < 0.05$). The

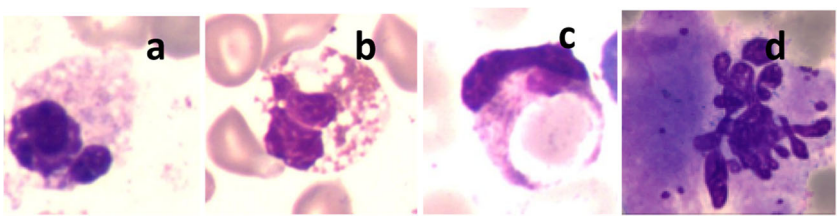

Fig. 2 Specific morphological dysplasias reported in the i(20q-) patients from a previous literature. **a** Hypogranulated and vacuolized neutrophils. **b** Hypogranular and vacuolized eosinophils. **c** Neutrophil erythrophagocytosis. **d** Deeply lobulated and hyperlobulated megakaryocytes

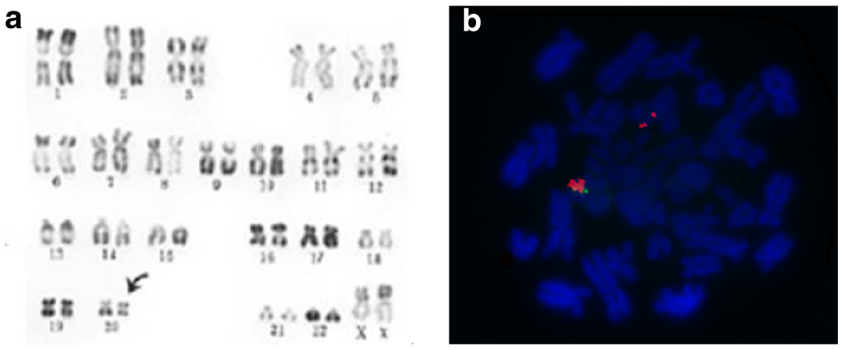

Fig. 3 **a** R-banded karyotype from a patient of 46,xx,i(20q-). **b** FISH result of patient using dual-color probes for 20q12 (green) and 20qter (red). Showing two symmetric red signals at both terminals of the chromosome i(20q-) but without any green signal between them, the other chromosome with two green and two red signals was a normal chromosome 20

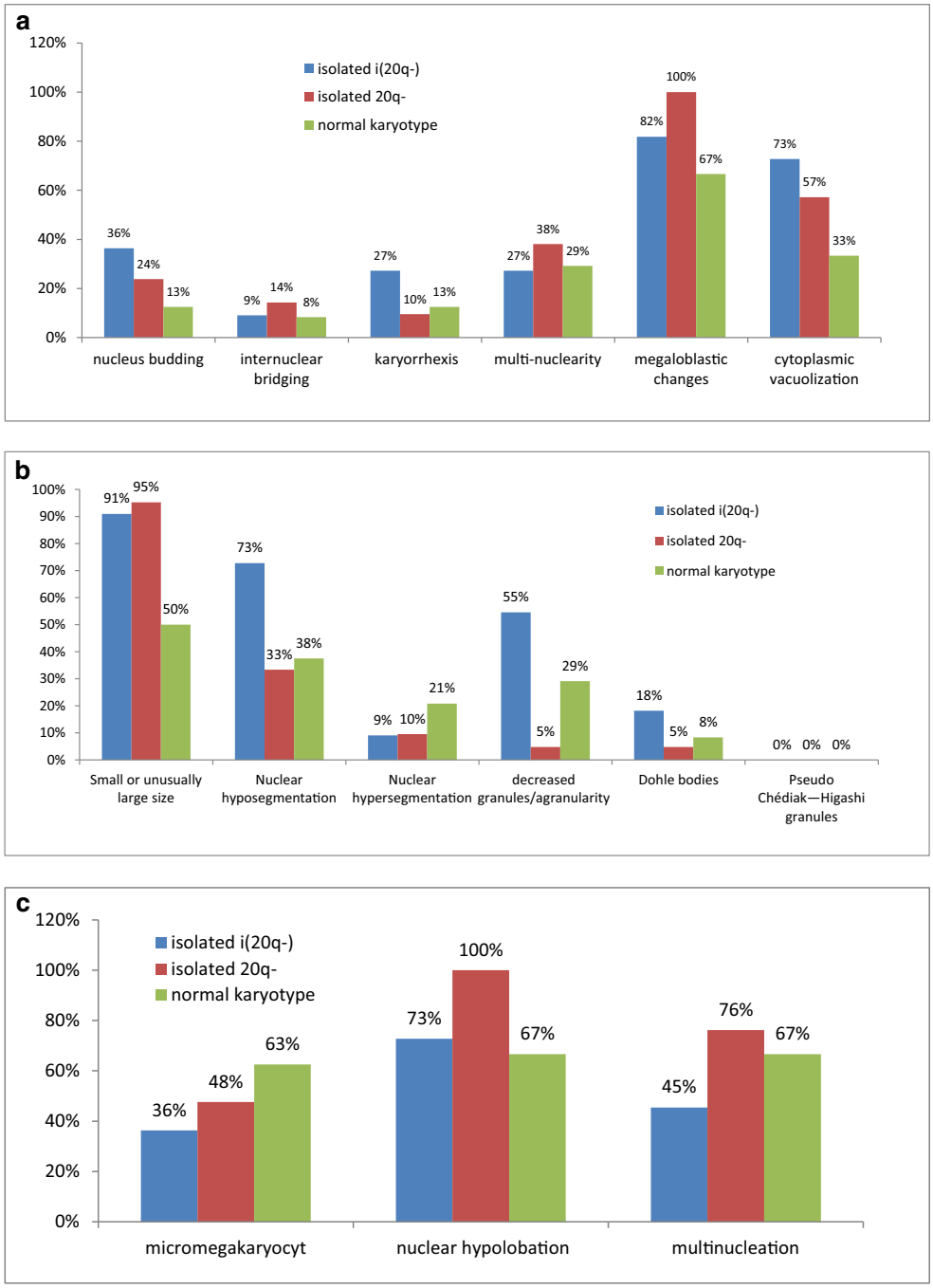

Fig. 4 The IMD-WHO in erythroid, myelocytic, and megakaryocytic lineage in MDS patients with isolated i(20q-), isolated 20q-, and normal karyotypes. **a** The incidence of cytoplasmic vacuolization in erythroid cells in the MDS patients with isolated i(20q-) was significantly higher than that in patients with normal karyotypes (8/11, 73% vs 8/24, 33%; $P < 0.05$). **b** The incidence of small or unusually large size in myeloid lineage in MDS patients with isolated i(20q-) was significantly higher than that in patients with normal karyotypes (10/11, 91% vs 12/24, 50%; $P < 0.05$). The incidence of nuclear hyposegmentation and decreased granules/agranularity in the MDS patients with isolated i(20q-) was higher than that in patients with isolated 20q- (8/11, 73% vs 7/21, 33%; 6/11, 55% vs 1/21, 5%; $P < 0.05$). **c** The incidence of nuclear hypolobation in megakaryocytic lineage in the MDS patients with isolated i(20q-) was lower than that in patients with isolated 20q- (8/11, 73% vs 21/21, 100%; $P < 0.05$). IMD-WHO, incidence of morphological dysplasia by 2017 WHO classification

incidence of Dohle bodies in the patients with isolated i(20q-) was numerically higher than that of the other two MDS group, respectively, but without significant difference ($P > 0.05$). The dysplasia of Pseudo Chédiak–Higashi granules was hardly found in these three MDS groups.

As shown in Fig. 4c, the nuclear hypolobation was consistently ranked as the primary dysplasia in megakaryocytic lineage in patients with isolated i(20q-), isolated 20q-, and normal karyotypes (73%, 100%, and 67%, respectively). But the incidence of nuclear hypolobation in isolated i(20q-) patients was significantly lower than that in isolated 20q- patients ($P < 0.05$). The incidence of other dysplasias in megakaryocytic lineage in isolated i(20q-) patients was numerically lower than that of the other two groups but without significant difference ($P > 0.05$).

Analysis of PDC-WHO in the erythroid, myeloid, and megakaryocytic lineages among MDS patients with isolated i(20q-), isolated 20q-, and normal karyotypes

The PDCs-WHO of these three lineage cells in MDS patients with isolated i(20q-), isolated 20q-, and normal karyotypes were plotted in Fig. 5:

As shown in Fig. 5a, for MDS patients with isolated i(20q-), isolated 20q-, and normal karyotypes, the most commonly detected dysplasia cells in erythroid lineage was megaloblastic changes (7.1%, 7.8%, 7.8% respectively); the next commonly detected was cytoplasmic vacuolization (5.9%, 5.3%, 4.9% respectively) in the three groups. As for the dysplasia of cytoplasmic vacuolization in erythroid cells, the PDC of the patients with isolated i(20q-) was significantly higher than that of the patients with normal karyotypes ($P < 0.05$). The PDC of nucleus budding in erythroid cells of the patients with isolated i(20q-) was numerically higher than that of the other two groups of MDS, but without significant difference ($P > 0.05$).

As shown in Fig. 5b, for patients with isolated i(20q-) and isolated 20q-, the most commonly detected dysplasia cell in myeloid lineage was small or unusually large size (8.1%, 7.8% respectively); yet for normal karyotype MDS patients, the most detected dysplasia cell was decreased granules/agranularity (5.7%). As for the dysplasia of small or unusually large size, the PDC of the patients with isolated i(20q-) was significantly higher than that of patients with normal karyotypes ($P < 0.05$). The PDC of nuclear hyposegmentation in patients with isolated i(20q-) MDS was significantly lower than that in patients with isolated 20q- MDS while significantly higher than that in patients with normal karyotype MDS, respectively (both $P < 0.05$). As for the dysplasia cell of decreased granules/agranularity, the PDC of the patients with isolated i(20q-) was significantly higher than that of patients with isolated 20q- ($P < 0.05$).

As shown in Fig. 5c, for patients with isolated i(20q-) and isolated 20q-, the most commonly detected dysplasia cell in megakaryocytic lineage was nuclear hypolobation (22.1%, 18.2% respectively); yet for normal karyotype MDS patients, the most commonly detected dysplasia cell was multinucleation (12.1%). As for the dysplasia of nuclear hypolobation, the PDC of the patients with isolated i(20q-) was significantly higher than that of the patients with normal karyotypes ($P < 0.05$).

Comparison of the dysplasia lineage (PDC-WHO ≥ 10%) in erythroid, myeloid, and megakaryocytic lineages among MDS patients with isolated i(20q-), isolated 20q-, and normal karyotypes

As shown in Fig. 6, the PDC-WHO ≥ 10% in erythroid lineage was found in 73%, 57%, and 38% of the patients with isolated i(20q-), isolated 20q-, and normal karyotypes, respectively, showing no statistical significance between the patients with isolated i(20q-) and the other two groups; the PDC-WHO ≥ 10% in myeloid lineage was found in 91%, 76%, and 67% of the patients of these three groups, respectively, without statistical significance. The PDC-WHO ≥ 10% in megakaryocyte lineage was found in 73%, 48%, and 42% of these three group patients respectively, showing no statistical significance between patients with isolated i(20q-) and the other two groups.

Comparison of the IMD and the PDC according to the type of specific morphological features reported in the publications among MDS patients with isolated i(20q-), isolated 20q-, and normal karyotypes

As shown in Fig. 7a, in the isolated i(20q-) cohort, the incidence of deeply lobulated and hyperlobulated megakaryocytes was ranked as the primary characteristic dysplasia (55%), which was much higher than that of MDS patients with isolated 20q- (19%) and normal karyotypes (17%) ($P < 0.05$). Moreover, the incidence of hypogranular and vacuolized eosinophils in isolated i(20q-) patients (27%) was also significantly higher than that in normal karyotype MDS patients (0%) ($P < 0.05$). In addition, the incidence of other specific morphological dysplasias reported in previous publications (hypogranulated and vacuolized neutrophils, neutrophil erythrophagocytosis) in patients with isolated i(20q-) was also numerically higher than that in patients with isolated 20q- and normal karyotypes in our present cohort, although no statistical significance was found among these three groups. These findings were generally consistent to previous reports.

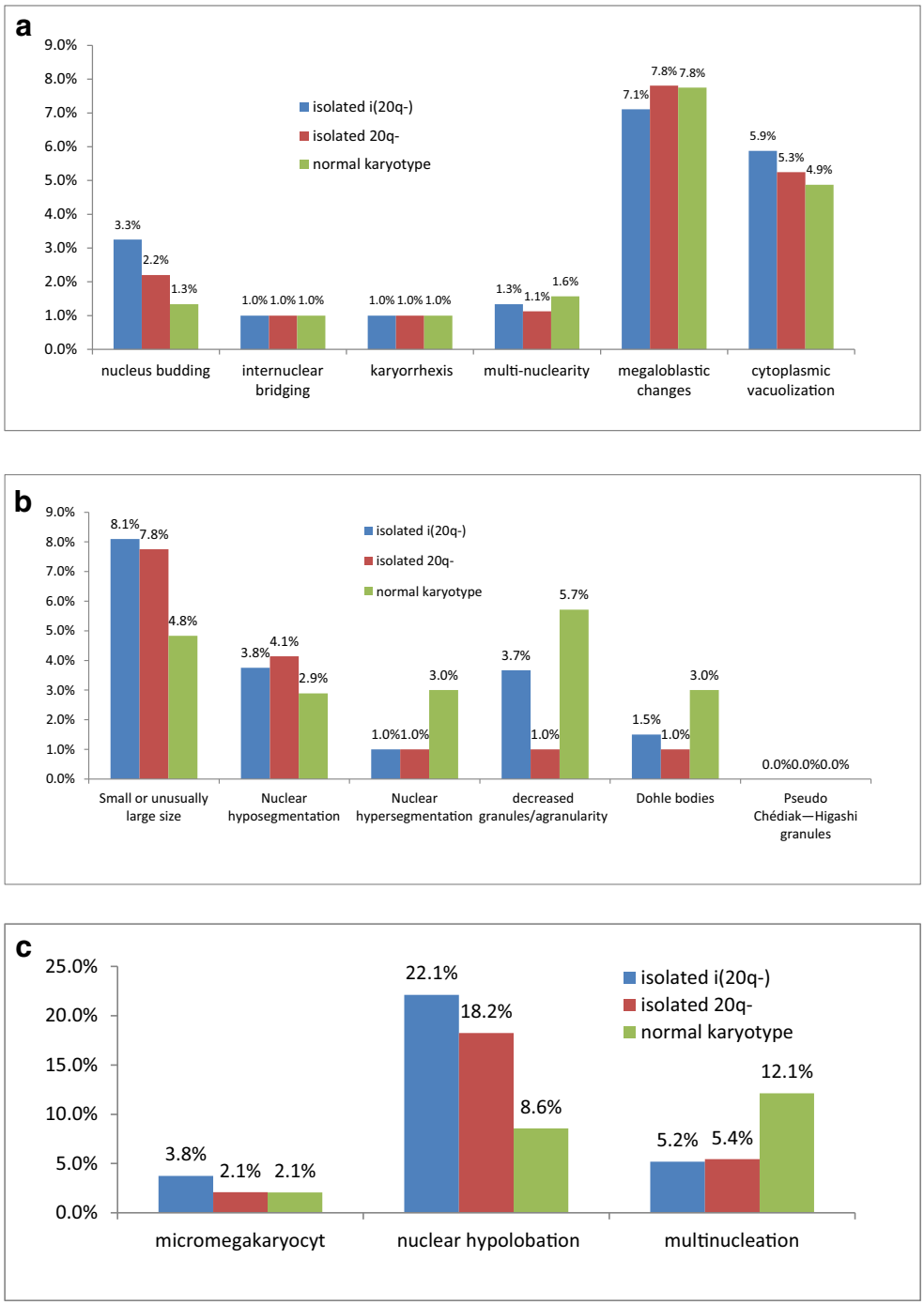

Fig. 5 The PDC-WHO of erythroid, myelocytic, and megakaryocytic lineages among MDS patients with isolated i(20q-), isolated 20q-, and normal karyotypes**. a** Among erythroid cells, the PDC of cytoplasmic vacuolization of the MDS patients with isolated i(20q-) was significantly higher than that of patients with normal karyotypes (mean 5.9% vs 4.9%; $P < 0.05$). **b** Among myeloid cells, the PDC of small or unusually large size in the MDS patients with isolated i(20q-) was significantly higher than that in patients with normal karyotypes (mean 8.1% vs 4.8%; $P < 0.05$); the PDC of nuclear hyposegmentation in MDS patients with isolated i(20q-) was significantly lower than that in patients with isolated 20q-, and significantly higher than that in patients with normal karyotypes (mean 3.8% vs 4.1%;, mean: 3.8% vs 2.9%; both $P < 0.05$). The PDC of decreased granules/agranularity of the MDS patients with isolated i(20q-) was significantly higher than that of patients with isolated 20q- (mean 3.7% vs 1.0%; $P < 0.05$). **c** Among megakaryocytic cells, the PDC of the nuclear hypolobation in the MDS patients with isolated i(20q-) was significantly higher than that in patients with normal karyotypes (mean 22.1% vs 8.6%; $P < 0.05$). PDC-WHO, percentage of dysplastic cells by 2017 WHO classification

As shown in Fig. 7b, as for the specific morphology of deeply lobulated and hyperlobulated megakaryocytes, the PDC of the patients with isolated i(20q-) (5.0%) was significantly higher than that of isolated 20q- (4.0%) and normal karyotype (1.0%) MDS patients respectively (both $P < 0.05$). The PDC of hypogranular and vacuolized eosinophils in patients with isolated i(20q-) (2.0%) was also significantly higher than that in normal karyotype (0.0%) MDS patients ($P < 0.05$).

Gene mutation analysis for the MDS patients with isolated i(20q-), isolated 20q-, and normal karyotypes

As shown in Table 2, MDS patients with isolated i(20q-) ($n = 2$), isolated 20q- ($n = 7$), and normal karyotype ($n = 13$) were subjected to gene mutation testing by next-generation sequencing (NGS). The incidence of mutation in 20q- MDS patients (8/9, 88.9%) was higher than that in normal karyotype

patients (7/13, 53.8%). Among the 20q- group, the incidence of mutation in isolated i(20q-) MDS patients (2/2, 100%) was higher than that in isolated 20q- MDS patients (6/7, 85.7%). Among all the 22 MDS patients, the gene mutations detected were U2AF1 (4/22, 18.2%), SF3B1 (4/22, 18.2%), NPM1 (3/22, 13.6%), FLT3-ITD (2/22, 9%), TET2 (2/22, 9%), and CSMD1, ASLX1, ETV6, NRAS, NOTCH1, etc. The mutations detected in MDS patients with isolated i(20q-) were U2AF1(1/2, 50%), CSMD1 (1/2, 50%), and SF3B1 (1/2, 50%); the mutations of U2AF1 and CSMD1 were detected in the same individual patient.

Discussion

From July 2002 to December 2017, 13 cases of isolated i(20q-) and 97 cases of isolated 20q- were detected among the inpatients and outpatients in our center. Eleven/thirteen (85%) of isolated i(20q-) patients were diagnosed as MDS, with the proportion much higher than that of isolated 20q- patients (21/97, 22%) ($P < 0.05$). As a type of common chromosome abnormality, isolated 20q- can be found in MDS, MPN, AML, CML, and so on and is nonspecific for MDS patients. Isochromosome 20q- (i(20q-)), as a rare reproducible chromosomal anomaly, was seldom reported because of the very low incidence, particularly for isolated i(20q-). In our study, isolated i(20q-) was found mostly in MDS patients, which was consistent with the literature report. Therefore, we assumed whether the isolated i(20q-) could be considered as a presumptive evidence of cytogenetic abnormalities for MDS. Based on this, in the present study, the morphological dysplasia features of these MDS patients with isolated i(20q-) were carefully studied according to the latest dysplasia standard by 2017 WHO classification, attempting to explore whether the MDS patients with isolated i(20q-) may exhibit specific morphological manifestations different from that of MDS patients with isolated 20q- and normal karyotypes. In addition, both isolated i(20q-) MDS patients and isolated 20q- MDS patients were older than the MDS patients with normal karyotype reflected by the median age data ($P < 0.05$).

The incidence of most morphological dysplasia in erythoid, myeloid, and megakaryocyte lineages described in the 2017 WHO classification did not differ from each other among the MDS patients with isolated i(20q-), isolated 20q-, and normal karyotypes. However, the cytoplasmic vacuolization in erythoid cells and small or unusually large size, nuclear hyposegmentation, and decreased granules/agranularity in myeloid cells were more commonly found in the patients with isolated i(20q-) than in those with isolated 20q- or with normal karyotypes. While in megakaryocytic lineage, the incidence of nuclear hypolobation in patients with isolated i(20q-) and normal karyotypes was statistically lower than that in patients with isolated 20q- respectively. These results indicated that morphological dysplasias in erythoid and myeloid lineage were more frequently detected in MDS patients with isolated i(20q-), and nuclear hypolobation in megakaryocytic lineage may be one of characteristics of isolated 20q- MDS patients.

As for the proportion of dysplasia cells in the bone marrow, the percentage of cytoplasmic vacuolization in erythoid cells, small or unusually large size, and nuclear hyposegmentation in myeloid cells and nuclear hypolobation in megakaryocytic lineage was higher in patients with isolated i(20q-) than that in patients with normal karyotypes, showing significant difference. As for the type of decreased granules/agranularity in myeloid cells, the percentage of such dysplasia cells in MDS patients with isolated i(20q-) was statistically higher than that in isolated 20q- patients. Thus, these morphological features mentioned above in three lineages contributed a higher degree

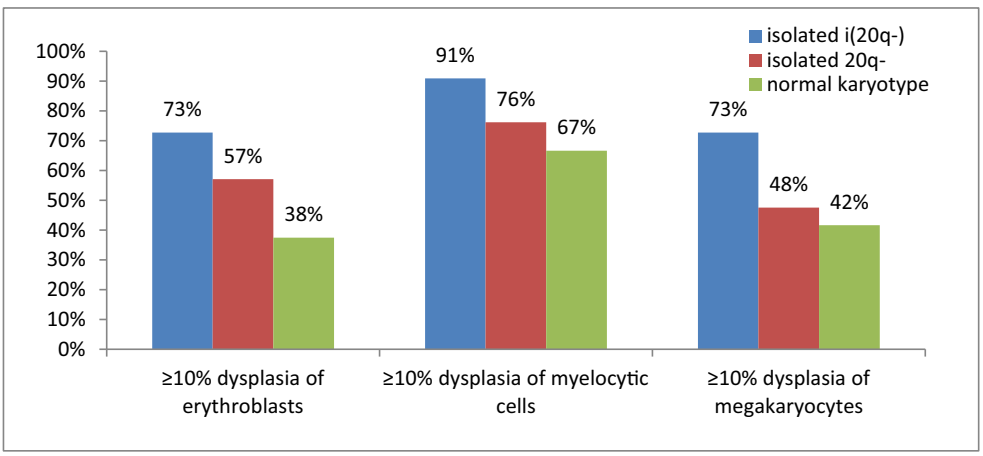

Fig. 6 Incidence of dysplasia lineage (PDC ≥ 10%) of the erythroid, myelocytic, and megakaryocytic lineages in MDS patients with isolated i(20q-), isolated 20q-, and normal karyotypes. The incidence of dysplasias (PDC ≥ 10%) of the MDS patients with isolated i(20q-) was numerically higher than that of patients with isolated 20q- and normal karyotypes in erythroid lineage (8/11, 73% vs 12/21, 57% and 9/24, 38%), myeloid lineage (10/11, 91% vs 16/21, 76% and 16/24, 67%), and megakaryocytic lineage (8/11, 73% vs 10/21, 48% and 10/24, 42%), respectively, but showing no statistical significance ($P > 0.05$)

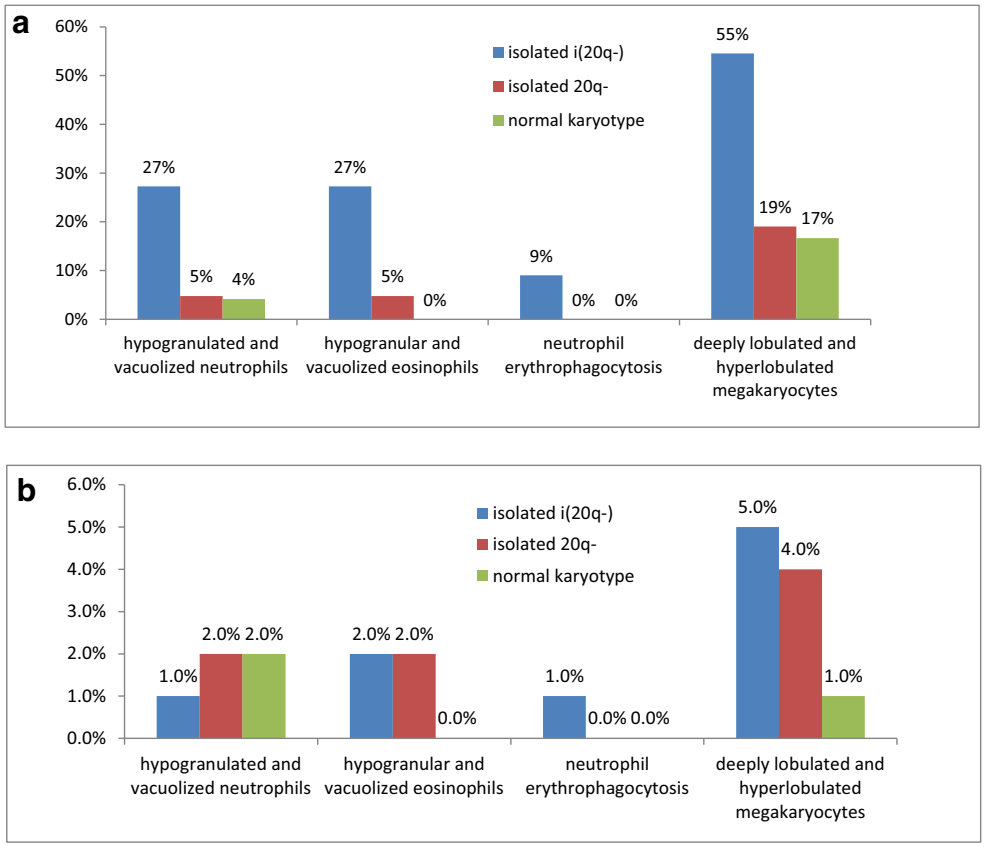

Fig. 7 Analysis of specific morphological features reported in the publications in MDS patients with isolated i(20q-), isolated 20q-, and normal karyotypes. **a** In the MDS patients with isolated i(20q-), the incidence of deeply lobulated and hyperlobulated megakaryocytes was significantly much higher than that in patients with isolated 20q- and normal karyotypes respectively (6/11, 55% vs 4/21, 19% and 4/24, 17%; both $P < 0.05$). The incidence of hypogranular and vacuolized eosinophils in the MDS patients with isolated i(20q-) was significantly higher than that in patients with normal karyotypes (3/11, 27% vs 0/21, 0%; $P < 0.05$). **b** The PDC of deeply lobulated and hyperlobulated megakaryocytes in the MDS patients with isolated i(20q-) was significantly higher than that in patients with isolated 20q- and normal karyotypes respectively (mean 5.0% vs 4.0% and 1.0%; both $P < 0.05$). The PDC of hypogranular and vacuolized eosinophils in the MDS patients with isolated i(20q-) was significantly higher than that in patients with normal karyotypes (mean 2.0% vs 0.0%; $P < 0.05$). PDC, percentage of dysplastic cells

of dysplasia of bone marrow cells in the isolated i(20q-) group than in the normal karyotype MDS group. These dysplastic features may reflect the complexity of the karyotypes. In future clinical practice, these dysplastic morphological manifestations may indicate the possibility of i(20q-).

As reported by François Mullier et al. [10], the MDS patients with i(20q-) may have distinct marrow morphological manifestations which are different from those with 20q-, including the presence of hypogranulated and vacuolized neutrophils and neutrophil erythrophagocytosis, detected with the specificity and sensitivity ranged between 65 and 75%, and the deeply lobulated and hyperlobulated megakaryocytes with specificity and sensitivity up to 70% and 85.7%, respectively. Therefore, they concluded that the presence of the above mentioned morphological manifestations in MDS may be a sign for possible i(20q-).

In this study, all of the specific morphological dysplasias reported by François Mullier et al. were entirely observed in

Table 2 The gene mutation test results of 21 patients with isolated i(20q-), isolated 20q-, and normal karyotype

	20q- group	Isolated i(20q-)	Isolated 20q-	Normal karyotype
Patients with detected mutation (*n*)	9	2	7	13
Patients with mutation (+) (*n*, %)	8 (88.9%)	2 (100%)	6 (85.7%)	7 (53.8%)
1 mutation (*n*, %)	6 (66.7%)	1 (50%)	5 (71.4%)	2 (15.4%)
2 mutations (*n*, %)	2 (22.2%)	1 (50%)	1 (14.3%)	4 (30.8%)
> 2 mutations (*n*, %)				1 (7.6%)

n number of patients

the isolated i(20q-) patients, and that the incidence was all numerically higher than that of isolated 20q- and normal karyotypes MDS patients. Furthermore, the incidence of deeply lobulated and hyperlobulated megakaryocytes in the isolated i(20q-) patients (55%) was much higher than that in isolated 20q- (19%) and normal karyotype (17%) MDS patients, respectively, showing statistical significance (both $P < 0.05$). These findings were consistent with those reported by François Mullier et al. Besides, the PDC of deeply lobulated and hyperlobulated megakaryocytes in patients with isolated i(20q-) (5.0%) was significantly higher than that in isolated 20q- (4.0%) and normal karyotype (1.0%) MDS patients respectively ($P < 0.05$). Therefore, the morphological manifestations of deeply lobulated and hyperlobulated megakaryocytes may be an important hint for the anomaly of isolated i(20q-). If these characteristic morphological manifestations (hypogranulated and vacuolized neutrophils, hypogranular and vacuolized eosinophils, neutrophil erythrophagocytosis) were observed simultaneously, it may indicate a serious possibility of chromosome abnormality of isolated i(20q-).

Currently, a majority of investigators deem the hematopoietic cell kinase (HCK) gene is associated with the presence of hypogranulated and vacuolized neutrophils. The gene located on 20q11.21 is a tyrosine kinase mainly expressed in myeloid cells, functioning to modulate granulocyte colony-stimulating factor dependent proliferation, migration and degranulation of myeloid precursors [11]. Under the condition of 20q-, the loss of HCK may occur due to the long arm deletion of chromosome 20. By contrast, in the case of i(20q-), the gene may be retained due to the replication of the residual long arm of chromosome 20, playing a pivotal role in the formation of hypogranulated and vacuolized neutrophils. Currently, the underlying genetic mechanism of neutrophil erythrophagocytosis and neutrophil platelet phagocytosis is not clarified yet [12–14].

The results of mutation test of this study indicated that the incidence of gene mutations in MDS patients with abnormal karyotype (20q- anomaly) was higher than that in normal karyotype patients. The incidence of gene mutations in isolated i(20q-) MDS patients might be higher than that in isolated 20q- patients, but this still needs more cases to confirm because of the limited cases of isolated i(20q-) in this study. Notably, the gene mutations of U2AF1, CSMD1, and SF3B1 could be detected in MDS patients with isolated i(20q-).

In conclusion, patients with isolated i(20q-) were mostly identified as MDS patients. The high frequency and high proportion dysplasia type of cytoplasmic vacuolization in erythoid cells and small or unusually large size in myeloid cells may be the morphological features of isolated i(20q-) MDS patients different from those of normal karyotypes MDS patients. The high frequency and high proportion dysplasia of decreased granules/agranularity in myeloid cells may be considered the features of isolated i(20q-) MDS patients distinguishable from those of isolated 20q- MDS patients. The morphological manifestations of deeply lobulated and hyperlobulated megakaryocytes and hypogranular and vacuolized eosinophils may provide the specific morphological implication for the anomaly of isolated i(20q-).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

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