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ASXL1 gene alterations in patients with isolated 20q deletion

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Deletion 20q is a recurrent abnormality in myeloid malignancies. In our previous study, we identified fusion of additional sex combs-like 1 (*ASXL1*) and teashirt zinc finger homeobox 2 genes in a patient with myelodysplastic syndrome. The objective of this study was to determine the frequency of *ASXL1* breakpoints in a cohort of 36 patients with deletion 20q as the sole cytogenetic aberration. A combination of molecular cytogenetic methods was used to confirm *ASXL1* gene alterations in 19 of the 36 patients and the determination of *ASXL1* gene changes in patients with deletion 20q revealed clinical and prognostic impacts.

Key words: ASXL1, FISH, deletion 20q, myelodysplastic syndrome, molecular cytogenetics

Deletion of the long arm of chromosome 20 [del(20q)] in bone marrow cells represents a common chromosomal abnormality associated with myeloid malignancies, in particular myelodysplastic syndromes (MDSs), acute myeloid leukemia (AML) and myeloproliferative neoplasms (MPNs) [1]. The deletion is most frequently interstitial and highly variable in size. As reported previously, proximal breakpoints are located in the 20q11.21–q12 region and distal breakpoints span from 20q13.13 to 20q13.33 and several commonly deleted regions have been identified in MDS, MPN and MDS/MPN patients [2]. Deletion 20q as the sole aberration is associated with a favorable outcome; such patients have considerably longer survival compared with other MDS patients [3]. In our previous study, we showed a fusion of additional sex combs-like 1 (*ASXL1*) and teashirt zinc finger homeobox 2 genes that resulted in an isochromosome of deleted 20q – ider(20q) in a patient with MDS [4]. *ASXL1* is located on 20q11.21 (30,946,155–31,027,122 forward strand hg19/GRCh37-Feb_2009) and mutations of this gene are generally associated with poor prognosis across the spectrum of hematologic malignancies as reviewed in Alvarez et al. [5]. Majority of the mutations are located in exon 12; however, rare mutations have been detected in the other exons of *ASXL1* [6].

Our objective was to determine the frequency of *ASXL1* alterations in del(20q) cases, to characterize breakpoints in the *ASXL1* gene using microarray techniques [array comparative genomic hybridization (aCGH)] and to evaluate the difference in survival between patients with and those without *ASXL1* alteration.

Patients and methods

Fluorescence in situ hybridization (FISH) using locus specific-probes for 20q11, 20q12 and 20q13.12 regions (Abbott, Des Plaines, IL, USA; Kreatech Diagnostics, Amsterdam, The Netherlands; MetaSystems, Altlussheim, Germany) was performed to confirm cytogenetically observed deletions of 20q in a cohort of 36 patients (27 males, 9 females, median age at diagnosis: 68 years) with hematologic disorders: MDSs (n=21), MPNs (n=10), non-Hodgkin lymphomas (n=2), acute myeloid leukemia (n=1), thrombocytopenia (n=1) and anemia (n=1). In all patients, deletion of 20q was the sole cytogenetic aberration, no other cytogenetic changes were found; however, in three patients a variant of del(20q), an isochromosome of deleted 20q, was detected. All patients provided informed consent for use of their samples for research purposes. Metaphase

Table 1. Clinical data and aCGH results in a cohort of eight patients with breakpoints in the *ASXL1* gene and with partial deletion of the 3′ end of the gene.

Pat. No.	Sex	Age	Diagnosis	aCGH findings	Survival (months)
1*	M	79	MDS	arr[GRCh37] 20pterp11.1(60770_25755060)x1,20p11.1q11.21(25805264_30954484)x3, 20q11.21q13.2(30960195_52045077)x1,20q13.2qter(52097649_62949120)x3	22
2	M	78	MDS	arr[GRCh37] 20q11.21q13.13(30948635_49457826)x1	50
3	M	75	Thrombocytopenia	arr[GRCh37] 20q11.2q13.13(31018764_49797610)x1	8
4	M	77	MDS	arr[GRCh37] 20q11.21q13.2(31006521_50759886)x1	7
5	M	64	MPN	arr[GRCh37] 20q11.21q13.13(31001393_49205106)x1	39+
6	M	63	MDS	arr[GRCh37] 20q11.21q13.13(31012919_49629926)x1	40+
7	M	64	MDS	arr[GRCh37] 8q11.21qter(48770702_143562095)x2 hmz,20q11.21q13.13(30954773_49507643)x1	50
8	M	74	MPN	arr[GRCh37] 20q11.21q13.2(31001594_51764010)x1	unknown

M: male; MDS: myelodysplastic syndromes; MPN: myeloproliferative neoplasms; *Patient with ider(20q); + Patient is still alive

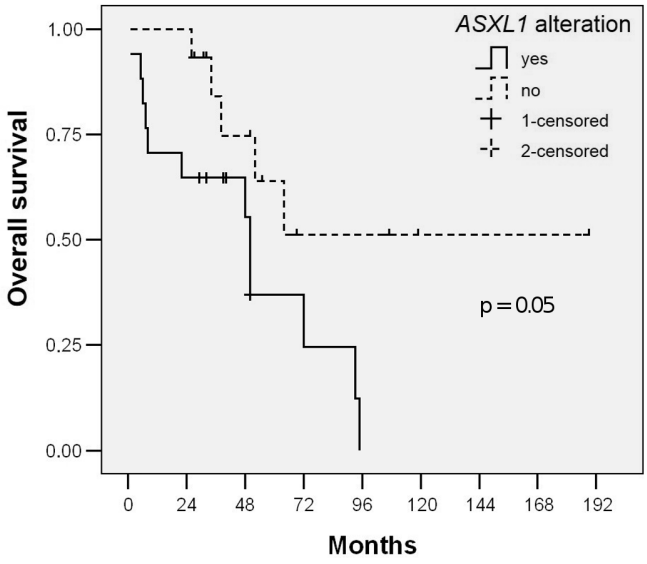

Figure 1. Kaplan-Meier survival curves for patients with and without *ASXL1* gene alterations. Kaplan-Meier survival curves proved significant differences (overall survival 48.6 and 119.0 months, respectively; p=0.05) between these two groups.

FISH mapping using a set of five bacterial artificial chromosome (BAC) probes (RP11-600A9, RP1-316I5, RP11-358N2, RP4-669H2 and RP5-823G15, BlueGnome, Cambridge, UK; Empire Genomics Buffalo, NY, USA) that target sequences in 20q11.21 and 20q13.2, along with a chromosome-20-specific centromeric probe (Kreatech Diagnostics) as a control, were used for determination of the breakpoints. BACs RP11-358N2 (chr20: 30,938,520–31,115,717) and RP11-600A9 (chr20: 30,927,178–31,082,065) completely cover the *ASXL1* gene.

aCGH [CytoChip Cancer 4x180K, CytoChip Cancer SNP 4x180K (Illumina, San Diego, CA, USA); SurePrint G3 Cancer CGH+SNP Microarray 4x180K (Agilent, Santa Clara, CA, USA)] was performed on DNA samples from bone marrow cells derived from eight patients with suspected partial deletions in *ASXL1* to characterize the breakpoints.

Kaplan-Meier survival analyses were performed using Mantel-Cox, Breslow and Tarone-Ware tests.

Results

According to the FISH results, three groups of patients were established: (1) 8 patients (22%; males only) had a proximal breakpoint in *ASXL1* with partial deletion of the gene; (2) 11 patients (31%; 9 males, 2 females) had complete deletion of *ASXL1*; and (3) 17 patients (47%; 10 males, 7 females) had no alterations in either copy of *ASXL1*, with the proximal breakpoint of the deletion located downstream of this gene. In summary, the *ASXL1* gene was altered (partially or completely deleted) in 19 of the 36 patients (53%; 17 males, 2 females). aCGH confirmed a high heterogeneity of the breakpoints in *ASXL1*, with the most common frequency in/downstream of the exon 4 and with partial deletion of the 3′ end of the gene (Table 1). Of the 19 patients with *ASXL1* alteration, 12 (63%) died and of the 17 patients with no such alterations, 5 (29%) died. Kaplan-Meier survival curves for the patient groups with and without *ASXL1* gene alterations showed significant differences (overall survival 48.6 and 119.0 months, respectively; p=0.05, this is a threshold value for statistical significance, Figure 1). No significant difference in age between these two groups was found (p=0.126).

Discussion

Deletion 20q is a recurrent cytogenetic abnormality frequently found in bone marrow cells of MDS patients and when present as a sole cytogenetic aberration, it represents a favorable outcome according to IPSS-R (Revised International Prognostic Scoring System; [7]). In our study deletion of 20q was the sole cytogenetic aberration; however, in three patients, a variant of del(20q), an isochromosome of deleted 20q – ider(20q), was detected. This isochromosome is a subtle rearrangement that is difficult to recognize using classical cytogenetics and the prognosis is unclear due to the small number of cases reported [8, 9]. Several mutations recently

discovered by new technologies often significantly affect the prognosis of MDS patients. *ASXL1* is the second most frequently mutated gene in MDSs after *TET2* [10]. Many studies have identified *ASXL1* gene mutations in myeloid malignancies as unfavorable prognostic indicators (reviewed in [5]). Schnittger et al. [11] reported associations of *ASXL1* mutations with male sex and older age in AML patients. Similarly, a male predominance was also found in our study, the group with *ASXL1* alterations comprised of 17 males and only 2 females, compared with 10 males and 7 females in the group without *ASXL1* alterations. However, we did not detect a significant difference in age between these two groups (p=0.126). *ASXL1* alterations are most often frameshift, nonsense or missense mutations. Missense mutations lead to a change in one amino acid of a protein, leaving the rest of the amino acid sequence unaffected. Frameshift and nonsense mutations lead to a premature stop codon, resulting in a truncated and usually non-functional protein [5]. Truncated ASXL1 proteins caused by frameshift or nonsense mutations were associated with a significantly worse overall survival, compared with wild type ASXL1 or ASXL1 harboring a point mutation, among patients with chronic myelomonocytic leukemia or MDS [12–15]. We assume that the same association (ASXL1 protein truncation and worse survival) occurs in patients with MDS and deletion 20q with breakpoints in/upstream of *ASXL1* causing partial/whole deletion of this gene. In our cohort of 36 patients with del(20q), the whole gene was deleted in 11 patients and partially deleted in 8 patients. The breakpoints in *ASXL1* gene were heterogeneous with high recurrence in/downstream of exon 4 and with loss of the 3′ end of the gene. We also found a shorter survival of patients with *ASXL1* partial/total deletion compared with patients with no *ASXL1* alterations (48.6 vs. 119.0 months; p=0.05). However, not only truncation of the gene but also haploinsufficiency for loss of the whole gene may contribute to the disease progression. Only a few studies have focused on the *ASXL1* role in patients with 20q deletion. Bacher et al. [16] detected *ASXL1* gene deletion by aCGH in 10 of 30 patients (33.3%) selected randomly from a cohort of 305 patients with MDS and del(20q); whole *ASXL1* deletion was found in nine cases and a partial deletion in one case. Similarly, Huh et al. [17] identified 20q deletions in 23 of 1162 patients with MDS, MPN or AML using single nucleotide polymorphism array (SNP-A) and of those, 10 harbored del(20q11.21), corresponding specifically to the *ASXL1* locus. The authors suggested that ASXL1 loss of function might contribute significantly to the pathogenesis of hematopoietic diseases. Our results support these findings; however, the number of cases with *ASXL1* deletion was higher in our cohort (53% of patients; complete deletion in 11 and partial deletion in 8 cases). Based on the results of our study we assume that total/partial *ASXL1* gene deletion contributes to worse prognosis in patients with del(20q). In all patients with the breakpoint in *ASXL1* gene the 3′ end of the gene and exon 12 were lost and we hypothesized that the expression of this gene was downregulated. These results correspond with published data that *ASXL1* mutations associated with myeloid neoplasms display a truncation in exon 12 [18–20] and that there is a loss of ASXL1 protein expression when *ASXL1* mutations are detected in myeloid neoplasms [20]. The *ASXL1* mutation status is important in patients with a low or intermediate risk of MDS, as the difference in survival is significantly different between patients carrying an *ASXL1* mutation and those with wild type *ASXL1*. Notably, *ASXL1* mutations did not seem to have a prognostic impact in patients with a higher risk of MDSs based on the French–American–British and/or World Health Organization classification [10, 13]. We also showed a significant difference in the survival of patients with del(20q) and low risk of MDS according to IPSS-R [7], between with and without *ASXL1* alteration. Different techniques are used to identify the *ASXL1* alterations, of which next generation sequencing is the most common followed by Sanger sequencing. In our cohort of patients, FISH using specific BAC probes was used to identify *ASXL1* alteration and aCGH was used to confirm all partial *ASXL1* deletions identified by FISH. We consider FISH using specific BAC probes to be a reliable and robust technique that can readily detect *ASXL1* alterations in routine analyses of del(20q) patients. Identifying such alterations in cases with a low risk of MDS may have a clinical and prognostic impact and may help identify those patients who need to be monitored more carefully and more frequently than those without this genetic alteration.

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