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Molecular cytogenetic characterization of acute myeloid leukemia and myelodysplastic syndromes with multiple chromosome rearrangements

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Background and Objectives. Multiple chromosome rearrangements (MCRs) are found in 5-10% of newly diagnosed patients with acute myeloid leukemia (AML) and 15-30% of patients with myelodysplastic syndromes (MDS). However, the initial causes of MCRs and the molecular mechanisms involved are largely unresolved. Nor are the karyotypic patterns well studied, mainly because of the difficulties of obtaining complete karyotypes by G-banding. In this study, we applied spectral karyotyping (SKY) and comparative genomic hybridization (CGH) to investigate further the resulting chromosome imbalances and rearrangements in AML and MDS bone marrow cells with MCRs.

Design and Methods. Bone marrow cells from 12 AML and 10 MDS patients with MCRs were collected at diagnosis and analyzed by G-banding, SKY and CGH. The patients' characteristics were also collected to pinpoint potential similarities and/or differences between the patients.

Results. Our results show that some MCRs seen in AML are similar to MCRs seen in MDS. These MCRs often result in chromosome loss of 5q, 7q and 17p and gain of chromosome 8.

Interpretation and Conclusions. The characteristics associated with MRCs include old age, previous exposure to radio- and/or chemotherapy and a short survival time. Probably, these patients should be distinguished from AML patients with primary chromosome rearrangements among other unbalanced chromosome rearrangements. In our experience, SKY and CGH facilitated this process.

Key words: AML, MDS, cytogenetics, SKY, CGH, FISH.

Acquired cytogenetic aberrations are detected by conventional karyotyping in 55-75% of newly diagnosed patients with acute myeloid leukemia (AML), providing unquestionable evidence that acute leukemia arises from clonal expansion of a malignant transformed progenitor cell.[1,2] Many karyotypic abnormalities are associated with specific disease subtypes, characteristic morphologic and immunologic profiles, and distinct prognostic implications. Direct involvement of many recurring translocations, inversions, and deletions in the leukemogenetic process is supported by molecular dissection and cloning of genes adjacent to translocation breakpoints.[3] However, 5%-10% of AML do not have leukemia-specific aberrations at diagnosis, but multiple chromosome rearrangements (MCRs) involving three or more chromosomes.[1,2] AML patients with MCRs have a poor prognosis and it is likely that some of these rearrangements contribute to disease progression.[1,2] In myelodysplastic syndromes (MDS), MCRs are more common; 15-30% of such patients carry MCRs at diagnosis and specific translocations are rare.[4] MDS with MCRs tend to progress to AML and to confer a poor prognosis.[5] The genetic constitutions of MCRs are not well known. One reason is difficulties in obtaining complete karyotypes. The final karyotypes are often incomplete, including various chromosome abnormalities of unknown origin, as well as deletions or gains of chromosomes. In this study, we applied spectral karyotyping (SKY) and comparative genomic hybridization (CGH) in order to investigate the chromosome constitutions of multiple chromosome rearrangements in AML and MDS. The samples had previously been analyzed by G-banding. SKY and CGH greatly improved the karyotypes, giving a much clearer view of the chromosome pattern

Table 1. Patients' characteristics.

Pt. no.	*Diagnosis**	*Year/Age°/Sex*	*Indication for radio/chemotherapy*	*Survival#*	*Spectral karyotype*
1	MDS UNS →AML M1	1998/67/F	essential thrombocythemia, 1986-1989 melphalan	3	46,XX,der(5)t(5;19)(q11.2;?)[3]/ 46,idem,t(6;14)(p21;q11~12)[4]/46,XX[3]
2	AML M0	2000/80/M		4	42~43,XY,-3,der(5)t(5;19)(q11.2;?),del(6)(p22), der(9)t(6;9)(p22;q34),der(11;15)(q10;q10),der(12)t(12;19)(p11;?), -17, 19,der(20)t(11;20)(q2?3;q13.3)[cp8]/ 73~75<3n>,idem,+4,+der(5)t(5;19)(q11.2;?),+8,+10,-17,+18, 19,+der(20)t(11;20)(q2?3;q13.3),+21,+22[cp8]/46,XY[7]
3	AML M1	1996/74/F	breast cancer, 1964 cyclophosphamide and radiotherapy	3	48,X,t(X;1)(p11;p11),t(1;22)(q31;q12), der(3)t(3;11)(q12;q21~23),del(5)(q21q31), der(6)(17pter_17p?::15q2?1_q11::14q32_q11::6p23_6qter), der(7)t(6;7)(p23;q22),+der(8)t(3;8)(q21;q24),-14,+15, ider(15)(q10) t(11;15)(q13;q?)x2,der(17)t(15;17)(q2?1;p1?),+22[6]/ 49, idem,+r(11)(?;?)[8]
4	AML M2	1997/83/M		6	50~57,XY,+Y,+2,t(2;10;18;17)(q22~23;q23~24;q12;q11~12)x2, der(3)t(3;11)(q2?5;q23),+4,der(5)t(5;18)(q13;q12),+6,+6, t(6;8)(q1?6;q2?3)x2,+8,+11,del(12)(p12),+13, der(17)t(15;17)(q2?1;p11),+21[cp24]
5	AML M2	1999/63/F		5	45,XX,der(2)t(2;5)(q23;q31),der(5)t(5;10)(q1?4;q22), der(10)t(2;10)(q23;q22),der(11)t(11;22)(q23~25;?), der(17)t(17;18)(p13;q21),-18, der(22)t(11;22)(q12;p11.2)dup(11)(q?;q2?4)qdp(11)(q23q25)[9]/ 45,idem, t(1;13)(q2?;q2?2)[2]
6	AML M0	1999/73/M		1	44,XY,der(3)t(3;5)(q25;q1?5),t(3;11)(q12~13;q1?3), der(5)t(5;?)(q1?1.2;?),del(7)(q2?2q3?),-17, der(18)t(18pter_18q11.2::19?::21q?_21qter), der(19)t(19;21)(q12;q?),-21, der(21)t(18;21)(q11.2;p11.2)[cp5]
7	AML M2	1997/77/F		6	45,XX,del(5)(q1?5q31),+11,+13,i(13)(q10),-16, der(17)t(16;17)(?p11;p1?1),-18[10]
8	AML M4	1997/61/F	lung cancer, 1994 radiotherapy	9	45,XX,del(5)(q14q32),r(11)(p15q25)qdp(q2?3;q25),-18[8]/ 46~47,idem,r(11)(p?q?)[2]
9	AML M5a	1999/74/M		7	46,XY,der(7)t(7;8)(q31;p11),del(8)(q23),t(12;13)(p13;q21), der(16)t(8;16)(q23;q23)[7]/47~48,idem,r(8)(p11q21)x2[cp7]
10	AML M5a	2000/5/F		8	47,XX,der(7)t(7;10)(p15;p14),+8, der(10)t(7;10)(p15;p14)del(10)(q22), der(16)t(10;16)(q22;p13)[7]/46,XX[4]
11	AML M5a	1999/23F	Hodgkin's lymphoma, 1998 MOPP/ABVD	CCR	46,XX,t(8;16)(p11;p13),der(10)t(8;10)(p11;p12)[3]/ 47,idem,+1,del(1)(p11)[3]/46,idem,+1,der(1;21)(q10;q10)[6]
12	AML M5a	1995/25/M	Hodgkin's lymphoma, 1992 MOPP/ABVD	CCR	48,XY,+8,t(11;19)(q23;p13),+der(19)t(11;19)(q23;p13)[9]
13	AML M4	1998/64/F	polycythemia vera, 1987-1998 ^{32}P and busulfan	1	50,XX,+der(3)del(3)(p?)del(3)(q?)x2,+der(3)del(3)(p?)del(3)(q?)x2, +der(3)del(3)(p?)del(3)(q?),der(5)t(5;14)(q22;q22),-7, der(14)del(14)(q22)ins(14;7)(q1?;?)[8]/ 51,idem,+9,del(9)(p11),del(9)(q11)[2]/46,XX[5]
14	MDS V →MDS-AML	1999/76/M		1	45,XY,del(4)(q2?3q28),der(5)t(5;7)(q21;q32), der(7)t(7;8)(q32;q11), der(8)t(8;18)(q11;q11.2),-18,del(20)(q11)[9]
15	MDS III →AML M1	1999/79/M		8	46,XY,dup(3)(q24q2?9),del(5)(q15q33),der(7)t(7;8)(q3?1;?), der(8)qdp(p12p2?)trp(q1?q21),t(9;12)(p21;p13)ins(12;7)(q21;?), der(16)t(11;16)(q21;q21)[cp9]/46,XY[2]
16	MDS III	1996/63/F		1	41~45,XX,del(4)(q13),+del(5)(q11.2q3?),der(5)t(5;17)(q1?1.2;q11), der(5)t(5;21)(q1?1.2;q11)dup(21)(q11.2q22),-7,der(13;14)(q10;q10),-17, -21,dup(21)(q11.2q22)[cp11]/46,XX[2]
17	MDS I/II	1996/70/F	rheumatoid arthritis, 1974-1996 methotrexate and chlorambucil	9	44,XX,der(5)t(5;17)(q11.2;q11),-7,-17[5]/ 44~45,idem,der(X)t(X;7)(p21;p1?),t(4;5)(q31;q?31)[cp8]
18	MDS III	1995/78/F		1	47,XX,del(5)(q15q31),+i(9)(p10),der(11)t(1;11)(p2?;p14~15), del(12)(q1?5q2?2)[8]/48,idem,der(7)t(3;7)(q2?5;q31~32),+i(9)(p10)[2]
19	MDS IVa	1998/54/F	non-Hodgkin's lymphoma, 1992-1998 CHOP, MIME, ENAD, KNOSPE	3	47~50,XX,der(9;21)(p10;q10),t(13;14)(q22;q13),+1~4der(21)[11]
20	MDS I	1997/87/M			46,XY,t(9;16)(p13;p13),del(20)(q11)[9]/ 45,idem,der(12)t(12;18)(p1?1;q1?),-18[2]/46,XY[2]
21	MDS III	1998/52/M	astrocytoma, 1995 radiotherapy	4	44,XY,-5,-7,der(17)t(5;17)(p11;p11)[7]/46,XY[2]
22	MDS III	2000/58/F		10	47,XX,+1,der(1;13)(p10;q10),der(2)t(2;7)(p2?1;p1?5), del(5)(q1?5q3?1),der(7)del(7)(p15)del(7)(q11.2),+8,-13,-15, der(16)t(7;16)(?;q2?2),der(17)t(15;17)(q1?;p11),+mar[2]/ 46,idem,-der(7)del(7)(p15)del(7)(q11.2)[8]

**According to FAB classification → transformed to; °year and the patients' age at time of investigation; #survival time in months. CCR: continuous complete remission; MOPP: mechlorethamine, vincristine, prednisone, procarbazine; ABVD: doxorubicin, bleomycin, vinblastine, dacarbazine; CHOP: cyclophosphamide, doxorubicin, vincristine and prednisone; MIME: methyl-GAG, ifosfamide, methotrexate, etoposide; ENAD: etoposide, mitoxantrone, cytosine arabinoside and dexamethasone; KNOSPE: intermittent chlorambucil/prednisone.*

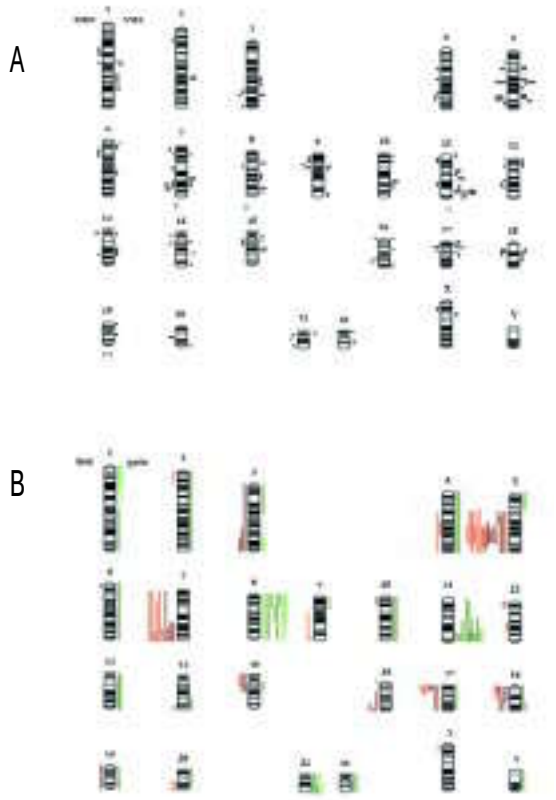

Figure 1. A. Distribution of the breakpoints, to the left in the MDS patients and to the right in the AML patients. A breakpoint was recorded once even if involved in more than one aberration in the same or related clone. Breakpoints that were identified by SKY but could not be assigned to a band are noted below the chromosomes (chromosome 7, 8, 11 and 19). B. Distribution of the chromosomal imbalances caused by unbalanced structural aberrations or numerical changes. To the right, gain of chromosome material: AML in dark green, MDS in light green. High-level amplifications are shown as bold lines (chromosome 8, 11 and 21). To the left, loss of chromosome material: AML in dark red, MDS in light red. When the same chromosome was involved in both numerical and structural rearrangements, the largest net imbalance was recorded.

formed by the MCRs. Furthermore, our results show that some of the MCRs seen in AML and MDS are related to each other. For these patients the net DNA imbalance often includes chromosome loss of 5q, 7q and 17p, and gain of chromosome 8. Probably these patients should be distinguished from AML patients with primary chromosome rearrangements among other unbalanced chromosome rearrangements. More precise karyotype data may be helpful in further defining subgroups of AML and MDS patients with MCRs that ultimately have clinical implications.

Design and Methods

Patients

Between January 1995 and June 2000, chromosome analyses were performed on approximately 140 newly diagnosed AML patients and 80 newly diagnosed MDS patients at the department of Clinical Genetics at the Karolinska Hospital. Fourteen of the AML patients (10%) and 13 of the MDS patients (16%) carried multiple chromosome rearrangements involving three or more chromosomes and were the subject of this study. However, five patients, two with AML and three with MDS, had to be excluded from the study as no metaphase cell suspension was available for the SKY analysis. The diagnosis and subtyping was performed according to the FAB classification.[6] A summary of the patients' characteristics is given in Table 1. Briefly, 9 of the 12 AML patients were above 60 years, two were in their twenties, and both had previously been treated with topoisomerase inhibitors for Hodgkin's disease, and one was 5 years old. The median age of the MDS patients was 68.5 years, the range being from 52 to 87. In three of the MDS patients the myelodysplasia later transformed to overt AML [patients # 1,14 and 15]. SKY analysis was performed in both phases for patient 1. In total, five AML patients and four MDS patients had previously been treated with either chemotherapy or radiation therapy for another malignancy.

Spectral karyotyping

Metaphase chromosomes were obtained from bone marrow cultures according to standard procedures and analyzed after G-banding. For SKY, slides were freshly prepared from chromosome suspension stored for up to four years in fixative (methanol:acetic acid 3:1) at -20°C. Pepsin treatment, hybridization and detection were carried out according to the protocol provided with the SKY-Paint™ hybridization and detection kit (Applied Spectral Imaging, Migdal Ha'Emek, Israel). Metaphase cells were captured using the SD200 spectral imaging system Spectral Cube (Applied Spectral Imaging), connected to a Zeiss Axioscop II fluorescence microscope. For each case, between 10-20 metaphases were analyzed using the SKY view software (Applied Spectral Imaging).

In situ hybridization

Standard fluorescence *in situ* hybridization (FISH) with a subtelomere probe for 5q was performed on metaphase slides from patients 3, 7, 8, 15, 16, 18 and 22 according to the manufacturer´s protocol (Vysis, Downers Grove, USA).

Comparative genomic hybridization

Comparative genonim hybridization (CGH) was performed on all bone marrow samples. Because no DNA was available from the original samples, single cells were isolated from metaphase cell suspensions by micromanipulation and amplified and labeled according to the single cell CGH protocol described by Klein *et al.*[7] In our experience, the protocol is optimal for one or two cells. In this study we used two cells for each CGH. As described in the results section, most patients had non-clonal chromosomal changes by SKY. Therefore, only chromosomal gains and losses dected by both SKY and CGH are presented in Figure 1B.

Results

Reclassification by SKY

In total, 101 structural aberrations were identified by SKY of which only 16 (16%) were possible to characterize to the same extent by G-banding. Nine of 11 (82%) chromosomes with additional material of unknown origin, 30 of 44 (68%) marker chromosomes, 9 of 16 (56%) deletions and 26 of 50 (52%) monosomies were reclassified by SKY as unbalanced translocations. For patient 6, the chromosome quality only allowed counting of the chromosomes by G-banding. By SKY, nine rearrangements could be identified. For two patients [patients 12 and 21] the karyotypes suggested by G-banding were verified. Finally, patient 1 had the same SKY karyotype also after the myelodysplasia had transformed to overt AML.

Breakpoints

The structural rearrangements resulted in a total of 88 and 61 breakpoints for AML and MDS respectively, located at 71 and 50 bands distributed along the chromosomes (Figure 1A). The most frequent breakpoints for AML were on 11q and 5q, representing 27.5% of all detected breakpoints and seen in 68% of the karyotypes. The most frequent breakpoints for MDS were on 5q representing 18% of all the detected breakpoints and seen in 80% of the karyotypes. Of a total number of 67 translocations, six were balanced: t(6;14) [patient #1], t(2;10;18;17) [patient #4], t(12;13) [patient #9], t(8;16) [patient #11], t(11;19) [patient #12], and t(13;14) [patient #16]. The remaining 61 (91%) were unbalanced by SKY analysis.

Chromosomal gains and losses

An overview of the CGH results is shown in Figure 1B. In addition to clonal chromosome aberrations, most patients also had non-clonal changes identified by SKY. Only chromosomal gains and losses detected by both SKY and CGH are presented in the figure. Numerous non-clonal rearrangements were detected in 4 patients (AML patients #2 and 6, MDS patients #15 and 16).

Eight AML and eight MDS patients had deletions on 5q, this being the most frequently lost chromosome region in both groups of patients (Figure 1B). For five AML and three MDS patients, the 5q- was the result of unbalanced translocations and not a terminal or interstitial deletion as earlier suggested by G-banding (Figure 2A). The remaining patients had interstitial deletions, verified by subtelomere FISH (data not shown). Furthermore, six 7q deletions were reclassified as unbalanced translocations by SKY (Figure 2B). In total, 3 AML and 3 MDS patients had lost 7q material [AML patients #3, 6 and 9, MDS patients #15, 18 and 22], and another four patients had lost one entire chromosome 7 [AML patient #13, MDS patients #16, 17, and 21]. Nine patients had lost a part or all of 17p [AML patients #2, 3, 5, 6, and 7, MDS patients #16, 17, 21 and 22] (Figure 1B). Additional chromosome 11q material was found in 7/12 AML patients [patients #2-5,7,8 and 15] and 1/10 MDS patients [patient #14], this being the most frequently gained chromosome region for AML (Figure 1B and 2C). Seven of the patients had structural rearrangements on 11q but only one translocation of the *MLL* gene was found [patient #12]. Instead, the other patients had gained additional copies of the *MLL* locus, varying between one and eight by locus-specific FISH (numbers of *MLL* signals are shown in brackets for each patient in Figure 2C). Furthermore, additional chromosome 8 material was seen in seven AML patients and two MDS patients [AML patients #2-4 and #9-12, MDS patients #15 and 22] (Figure 1B).

Discussion

Five to ten percent of AML and 15%-30% of MDS have multiple chromosome rearrangements involving three or more chromosomes. These aberrations are often considered random and non-specific and may be present at diagnosis or at disease progression. As a group, patients with MCRs at diagnosis have a relatively poor prognosis.[1] However, the genetic constitutions of MCRs are not well known and it is therefore possible that modern cytogenetic methods such as SKY and CGH will add valuable information that could ultimately result in subgrouping these patients with clinical implications on choice of treatment and prediction of prognosis. In this study, we applied spectral karyotyping and comparative genomic hybridization to bone marrow

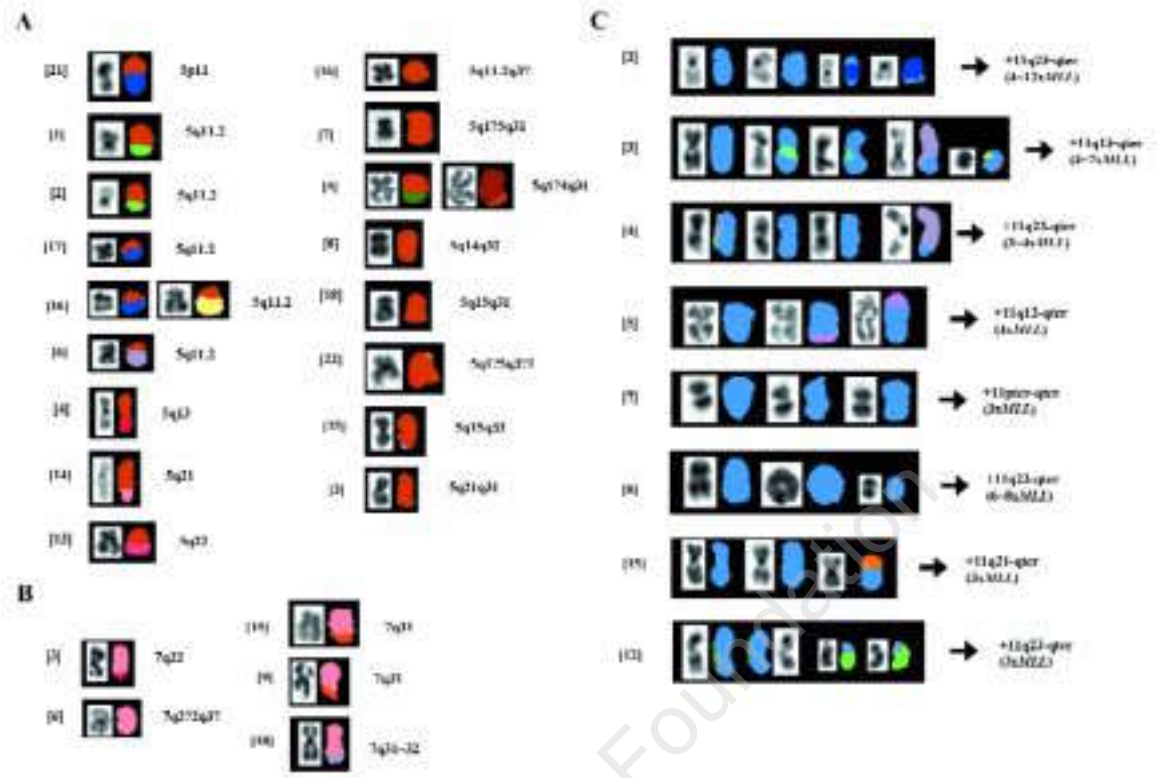

Figure 2. Illustration of the chromosome alterations on 5q (A), 7q (B) and 11q (C), resulting in chromosome loss of 5q and 7q, and gain of 11q. Patients' numbers are shown to the left. The derivative chromosomes are shown in the inverted DAPI display and in the SKY classification colors.

cells from 12 AML and 10 MDS patients with MCRs in order to investigate further the resulting chromosome imbalances and rearrangements. We summarized the patients' characteristics in an attempt to identify potential similarities and differences between the patients (Table 1).

It has previously been reported that MCRs are more common among older patients and patients with secondary AML or treatment-induced AML; our study adds further support to this finding. Nine of our patients had previously received chemotherapy or/and radiotherapy (Table 1). A broad range of agents and ionizing radiation may disrupt chromatin and lead to apoptosis if allowed to proceed to completion.[8] When interrupted prematurely, these signal pathways may produce translocations and thus allow the cell to escape its apoptotic fate, e.g. alkylating agents induce AML primarily by causing unbalanced chromosome aberrations, preferentially on chromosomes 5 and 7.[8,9] Loss of chromosome 5q material was seen in 16 patients, being the most frequent chromosome loss for both patient groups, followed by loss of chromosome 7q (Figure 1B). Interestingly, for eight patients the 5q loss was a result of unbalanced translocations rather than terminal deletions. Indeed, no terminal deletion was found. Instead the remaining eight patients had interstitial deletions on 5q. Although 5q deletions are common in AML, the breakpoints vary among patients. Identification of the deleted region is important because it may contain a putative tumor suppressor gene(s). Whether 5q- translocations have any other consequences beside the resulting loss of 5q needs to be further investigated. Six of the 5q translocations identified in our material involved band 5q11.2. It is possible that molecular cloning of the responsible gene(s) will be facilitated by identifying translocations on 5q. Furthermore, 9 patients had lost a part or all of 17p. It is possible that p53 on 17p13 is the target for some or all of these deletions. It has previously been reported that mutations with loss of heterozygosity of p53 are common in therapy-related MDS and AML after exposure to alkylating agents

and significantly associated with deletion or loss of 5q, a complex karyotype, and a poor prognosis.[10] All our patients with 17p deletions in their material also had 5q deletions. None of the patients with 17p- and 5q- reached complete remission, which further supports previous findings that this patient group has an extremely poor prognosis.

It has been suggested that ionizing radiation and environmental agents operating throughout life at a very low background level may be involved in the pathogenesis of *de novo* MDS, and that with increasing age this may ultimately lead to the development of overt AML.[11] In our study, three MDS patients [patients #1, 14 and 15] developed AML within the six months after the cytogenetic analysis presented here had been performed. Patient 1 had the same karyotype also after transformation to AML. Interestingly, additional chromosome 11q material was more frequently found in the AML patients than in the MDS patients. In total, one MDS and seven AML patients had gained additional copies of the *MLL* gene. Recently, several groups have reported on *MLL* copy number changes in AML and MDS suggesting that not only translocations but also acquired copies of the *MLL* gene are associated with leukemogenesis.[12-15] However, it is possible that MLL is merely a linked passenger to another, perhaps more biologically significant, gene in some or all of the amplification events seen in the material from our patients and other patients.

In our material we identified two cases with primary reciprocal translocations, one t(8;16)(p11;p13) [patient #11] and one t(11;19)(q23;p13) [patient #12], along with other unbalanced rearrangements. For patient 11, the t(8;16) was missed by G-banding. For some primary translocations additional aberrations do not seem to influence the prognosis significantly.[16] It is therefore important to distinguish these patients from other patients with MCRs. Both our patients with primary reciprocal translocations were in their twenties and had previously been treated with topoisomerase inhibitors for Hodgkin's disease. Inhibitors of topoisomerase II are capable of inducing DNA breaks at preferred positions in breakpoint cluster regions of genes such as the *MLL* gene (11q23) and the *CBP* gene (16p13.3).[17,18] Both patients obtained complete continuous remission and are still alive after 2 years (patient #11) and 6 years (patient #12). In sharp contrast, all the other patients in this study died within one year.

In conclusion, our results show that some MCRs seen in AML are similar to MCRs seen in MDS. They often include chromosome loss of 5q, 7q and 17p and gain of chromosome 8. The characteristics associated with MCRs include old age, previous exposure to radio and/or chemotherapy and a short survival time. Probably these patients should be distinguished from AML patients with primary chromosome rearrangements among other unbalanced chromosome rearrangements. In our experience, SKY and CGH facilitated this process. More precise karyotype data may be helpful in further defining subgroups of AML and MDS patients with MCRs that could ultimately have clinical implications.

Contributions and Acknowledgments

CL, MN, and EB designed the project. CL performed the analyses and wrote the paper. AP and MB acted in an advisory capacity. All authors approved the final version of the manuscript.

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Disclosures

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Manuscript processing

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Potential implications for clinical practice

The resolution of MCRs is substantially improved by SKY and CGH. More precise karyotype data may be helpful in defining subgroups of AML and MDS patients with MCRs that have clinical implications.

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