Univerzita Karlova v Praze

1. lékařská fakulta

Autoreferát disertační práce

Molekulárně cytogenetická analýza aberací chromosomu 11 u hematologických malignit

Molecular-cytogenetic analysis of chromosome 11 aberrations in hematological malignancies

RNDr. Iveta Šárová

Praha 2012

Doktorské studijní programy v biomedicíně

Univerzita Karlova v Praze a Akademie věd České republiky

Obor: Molekulární a buněčná biologie, genetika a virologie

Předseda oborové rady: Prof. RNDr. Stanislav Zadražil, DrSc.

Školicí pracoviště: Cytogenetické oddělení ÚHKT

Školitel: RNDr. Jana Březinová, Ph.D.

Konzultant: Prof. Ing. Kyra Michalová, DrSc.

Oponenti:

……
……
……

Autoreferát byl rozeslán dne: .....

Obhajoba se koná dne: ……. v ……. hod.

kde …….....

S disertací je možno se seznámit na děkanátě 1. lékařské fakulty Univerzity Karlovy v Praze.

Molecular-cytogenetic analysis of chromosome 11 aberrations in hematological malignancies

Ph.D. thesis summary

CONTENTS

Souhrn

Změny chromosomu 11 patří mezi časté cytogenetické nálezy u hematologických malignit. Zlomové místo se na tomto chromosomu obvykle nachází v oblasti 11q23.3, v proto-onkogenu *MLL*. Vzácně se zlomy vyskytují i v jiných oblastech, což svědčí o účasti i dalších genů. V naší studii jsme se zaměřili na identifikaci zlomů a oblastí amplifikace/delece na tomto chromosomu v buňkách kostní dřeně/periferní krve nemocných s nově diagnostikovanou akutní myeloidní leukémií (AML).

Potvrdili jsme, že změny chromosomu 11 jsou často se vyskytujícím cytogenetickým nálezem u dětských i dospělých nemocných s AML. Identifikovali jsme množství rekurentních i náhodných zlomových míst (rekurentní v genu *MLL* (11q23.3) a *NUP98* (11p15.4) a další potencionálně nenáhodné zlomy v oblastech 11p13, 11p12 a 11q13.2). Poukázali jsme na několik nových kandidátních genů s možnou úlohou v patogenezi AML. Vyhodnotili jsme nejčastěji deletované a duplikované/amplifikované oblasti a vybrali kandidátní tumor supresorové geny a onkogeny. Dokázali jsme, že změny chromosomu 11 jsou důležitým prognostickým markerem. Z klinického hlediska lze nemocné rozdělit do dvou skupin: pacienti s přestavbou *MLL* genu a pacienti s ostatními změnami chromosomu 11 bez ohledu na lokalizaci zlomu. Oproti nemocným s přestavbou genu *MLL* jsme prokázali spojitost ostatních změn chromosomu 11 se starším věkem nemocných, komplexním karyotypem, nebalancovanou změnou (na 11p v důsledku ztráty genetického materiálu a na 11q obvykle ziskem) a velmi nepříznivým klinickým průběhem s krátkou dobou přežití. FISH skríning zaměřený na detekci nejčastějších aberací u AML se jako rychlá analýza přítomnosti patologických nálezů spojených s nepříznivou prognózou ukázal velmi přínosný u pacientů s nedostatkem dělících se buněk a pro detekci kryptických přestaveb *MLL* genu.

Studium zlomových míst a oblastí amplifikace/delece na chromosomech slouží nejen ke klinické stratifikaci nemocných do prognostických skupin, ale je i nezbytným podkladem pro identifikaci genů, které asociují se vznikem a progresí nádorů. Molekulární a funkční analýzy těchto genů a jejich produktů nám pomáhají porozumět patogenezi maligní transformace, případně i lékové rezistence. Významně přispívají také k vývoji nových terapeutik a individuální léčby pro nemocné.

Summary

Chromosome 11 abnormalities are found in many hematological malignancies. In acute myeloid leukemia (AML), a proto-oncogene *MLL* (11q23.3) is frequently altered. However, rearrangements of other regions of chromosome 11 have been reported. Therefore, we have identified and characterized the chromosome 11 breakpoints and common deleted and amplified areas in the bone marrow or peripheral blood cells of newly diagnosed patients with AML.

Chromosome 11 abnormalities were proved as a frequent chromosomal change found in both adult and childhood newly diagnosed AML patients. Many recurrent and random breakpoints were identified (recurrent in the *NUP98* gene (11p15.4) and *MLL* (11q23.3), and three possibly nonrandom in chromosomal bands 11p13, 11p12 and 11q13.2). We notified new possibly significant genes in the development of AML. The most common deleted, duplicated/amplified regions and candidate tumor suppressor genes and oncogenes were determined. The potential involvement and contribution of affected areas of chromosome 11 to the malignant transformation process was discussed. The chromosome 11 changes were assessed as an important prognostic marker. From the clinical point of view, the chromosome 11 abnormalities can be stratified into two relatively homogenous groups: patients with *MLL* rearrangements and patients with other chromosome 11 changes. Unlike the patients with *MLL* rearrangements, the patients with other chromosome 11 changes are usually older, with complex karyotype, unbalanced aberrations and very poor clinical outcome. FISH screening focused on the most frequent aberration in AML was proved to be very helpful as a rapid detection of pathological clones with unfavourable prognosis and in case of dividing cells lack and cryptic *MLL* gene rearrangement.

In conclusion, molecular analyses of chromosomal breakpoints and amplified or deleted areas are very important not only for the patient stratification into specific prognostic and clinical subgroups but also for the identification of genes involved in tumor pathogenesis. Further investigation of the affected genes and their protein products will improve our understanding of the oncogenesis of AML and could be clinically applied for the designation of more effective therapeutic approach.

1. Introduction

1.1 Chromosome 11

Chromosome 11 represents approximately 4 to 4.5% of human genome (about 134.5 Mb in size, including the centromere) and belongs to the most gene and diseases rich chromosome. Until now, 1524 protein-coding genes have been identified on the chromosome 11 (Taylor et al. 2006).

Chromosome 11 has an important role in the history of molecular genetics. In the chromosomal band 11p15.5, the gene encoding β-hemoglobine is located. One nucleotide substitution leads to a defective protein and hematological disease known as a circle cell anemia (name according to the morphology of affected erythrocytes). The association of disease with abnormal protein was revealed by Linus Pauling for the first time in 1949.

Nowadays, the chromosome 11 abnormalities are believed to be at the origin of more than 100 congenital and malignant diseases for example Beckwith-Wiedeman syndrome, Emanuel syndrome, Jacobsen syndrome, Wilms tumor, hematological malignancies atd.

1.2. Molecular basis of hematological malignancies

The development and progression of hematological malignancies is based on the accumulation of genetic mutations and epigenetic changes in hematopoietic progenitor cells that alter normal cell processes such as cell growth, proliferation, and differentiation (Hanahan and Weinberg 2011). Some of these genetic alterations are random and have no diagnostic, prognostic or therapeutic significance. On the other hand, some others are recurrent with a specific occurrence in particular type of neoplasia and have important clinical applications (Mitelman 2000).

The typical genetic changes in neoplasia are unbalanced and balanced aberrations (Sandberg and Meloni-Ehrig 2010). From the cytogenetic point of view, the unbalanced changes can be presented as a lost of genetic material (monosomy or deletion which usually involved tumor suppressor) or gain of genetic material (trisomy, polyploidy, duplication or amplification usually including proto-oncogenes). Balanced rearrangements can be observed in the form of reciprocal translocation, inversion or insertion and gene structure and/or expression are modified. Not only protein-coding genes but also genes for miRNA can be altered by un/balanced rearrangements (Cho 2007).

1.3. Chromosome 11 changes in hematological malignancies

In hematological malignancies, chromosome 11 long arm abnormalities are more frequent than short arm changes. Breakpoints on the long arm are typically localized to chromosomal band 11q23.3, altering the proto-oncogene *MLL* (myeloid/lymphoid leukemia). *MLL* aberrations occur in 5-10% of acute leukemias including pediatric, adult and therapy-related hematological malignancies of both lymphoid and myeloid lineage (Meyer et al. 2009). However, the frequency may be higher because some *MLL* gene rearrangements have cryptic character (Park et al. 2008). The *MLL* translocations are known for their great variability caused by a number of different partner genes and for a diversity of mechanisms generating malignant *MLL* fusion gene. Approximately 85 different *MLL* rearrangements have been recently published. In more than 60 of these rearrangements, the concrete *MLL* partner gene has been identified; the most common partner genes are *AFF1* (4q21), *MLLT3* (9p22), *MLLT10* (10p12), *MLLT4* (6q27), *MLLT1* (19p13.3), and *ELL* (19p13.1) (Meyer et al. 2009). AML with 11q23 rearrangements, with the exception of t(9;11), are characterized by poor prognosis and negative clinical outcome (Marchesi et al. 2011).

Another mechanism of interrupting the *MLL* gene is a partial tandem duplication (PTD). The PTD of the *MLL* gene has been described in about 6 – 10% of adult patients with AML with normal karyotype and in most cases with trisomy 11 as a sole cytogenetic abnormality (Whitman et al. 2001, Mrozek et al. 2007). The partial duplications of the *MLL* gene usually include exones 2 through 8. Surprisingly, the PTD has been observed also in healthy population but in quantitatively lesser extent and/or includes series of untypical exones (Basecke et al. 2006). In rare cases, the duplication is nontandem due to insertion of DNA from another chromosome. Such partial nontandem duplications (PNTD) are results of multiple different rearrangements which consist of aberrations like *MLL* PTD and fusion of the *MLL* gene with other gene. That unusual complex rearrangement can simulate a simple translocation (Whitman et al. 2001).

Other identified altered genes in 11q are as follows *CCND1* (11q13.3), *ATM* (11q22), rarely *DDX10* (11q22-q23), *GAB2* (11q14.1), *MIR100* (11q24.1), and *NUMA1* (11q13).

On the short arm, the *NUP98* (nucleoporin 98 kDa) gene in chromosomal band 11p15.4 has been described as rarely altered. *NUP98* encodes a nucleoporin protein that normally mediates the transport of RNAs and proteins across the nuclear membrane (Iwamoto et al. 2010). *NUP98* belongs to the group of promiscuous genes involved in leukemic

chromosomal translocations and a total of 25 different fusion partners (often homeobox genes) have been reported (La Starza et al. 2009). Rearrangements in *NUP98* are rare and occur in various primary and therapy-related hematological diseases (Wang et al. 2010, Hollink et al. 2011). Therefore, it is difficult to define the prognostic impact of the various *NUP98* fusion genes and translocations. However, *NUP98* rearrangements seem to be more often associated with a poor outcome (Hollink et al. 2011).

Other identified altered genes in 11p are as follows *LMO1* (11p15) and *LMO2* (11p13).

However, for many other commonly rearranged regions of chromosome 11, the altered genes remain to be identified. In general, the chromosome 11 abnormalities have been described in many hematological malignancies, most often in acute myeloid leukemia (AML).

1.4. Acute myeloid leukemia (AML)

Acute myeloid leukemia is heterogeneous hematopoietic tissue disorder diagnosed in about 80% to 85% of all acute leukemias in adults. There are two classifications: FAB (French-American-British) and WHO (World Health Organization) used for subtyping. An abnormal karyotype is observed in 50-60% of AML cases (Mrózek et al. 2004, Marchesi et al. 2011). Many recurrent structural and numerical cytogenetic abnormalities associated with AML have been reported. In general, the most frequent AML abnormalities involve chromosomes 5 (deletion of long arm), 7 (deletion of long arm/monosomy 7), 8 (trisomy 8), 11 (*MLL* gene alterations), and 17 (deletion of tumor suppressor *TP53* gene) (Mrózek et al. 2008, Marchesi et al. 2011). Complex chromosomal aberrations (CCAs) defined as the presence of three or more chromosome abnormalities constitutes 10% to 15% of all AML patients (Babická et al. 2007, Mrózek et al. 2008).

Some specific structural and numerical cytogenetic aberrations were established as diagnostic markers for specific AML subtypes and as independent prognostic factors for complete remission attainment, relapse risk, and overall survival (Mrózek et al. 2007, Marchesi et al. 2011). Patients are classified into following three risk categories: favorable, intermediate and unfavorable. AML with translocations t(15;17)(q22;q21), t(8;21)(q22;q22) and inversion inv(16)(p13q22) are assigned to the favorable group, abnormalities of chromosomes 5, 7, 11, and 17 or complex chromosomal aberrations are mostly associated with unfavorable outcome. The prognosis of trisomy 8 remains controversial and is

classified as intermediate or unfavorable. Patients without cytogenetic aberration are in intermediate risk group (Mrózek et al. 2007, Marchesi et al. 2011).

First examination at the time of diagnosis is conventional cytogenetics. Unfortunately this technique is limited by factors such as cells proliferation, chromosomal morphology, extent of pathological clone with chromosomal aberration and resolution of methods. Therefore, some cryptic aberration can be missed. In these cases, FISH analysis is a suitable informative supplement as same as in patients where mitoses for karyotyping are not present.

1.4.1. Childhood AML

Childhood AML contains only 15 – 20% of all childhood acute leukemias. An abnormal karyotype is proved in almost 80% of AML childhood cases (Mrózek et al. 2004, Manola 2009). Children with AML can be divided into two groups.

Younger children are often diagnosed as AML FAB subtypes M4, M5 and M7 (usually children with Down syndrome). The most common chromosomal aberrations are rearrangements of the *MLL* gene (11q23.3) (Manola 2009). Balgobind et al. (2009) proved better clinical outcome in children with translocation t(1;11)(q21;q23), whilst t(6;11)(q27;q23), t(10;11)(p12;q23) and t(10;11)(p11.2;q23) have been associated with unfavorable prognosis.

Older children are usually diagnosed with AML FAB subtype M0, M1, M2 and M3. Their cytogenetic findings are more similar to that detected in adult AML patients (Mrózek et al. 2004). One of the common cryptic translocation, that seemed to be specific only for children, is t(5;11)(q35;p15) (fusion gene *NUP98-NSD1*). It has been proved in de novo childhood AML with apparently normal karyotype or with deletion del(5q) as a sole abnormality (Jaju et al. 2001). However, later it has been observed also in adult AML patients (Casas et al. 2003).

In spite of intensive chemotherapy, the half of AML childhood patients relaps and die. Therefore, the development of targeted therapy with minimal destruction of health tissue is very essential.

2. Aims of study

Chromosome 11 abnormalities are found in many hematological malignancies, frequently in acute myeloid leukemia (AML). Therefore, we focused our study on identification and characterization of the chromosome 11 breakpoints and common deleted/amplified areas in the bone marrow/peripheral blood cells in newly diagnosed patients with the diagnosis of AML.

The aims of our study were:

- to define chromosome 11 abnormalities in the bone marrow or peripheral blood cells of adult and childhood patients with newly diagnosed AML at the molecular-cytogenetic level

- to identify the locations of the breakpoints and commonly affected areas of deletions and duplications/amplifications, and to uncover the genes possibly involved in AML pathogenesis.

- to determine the potential association of identified chromosome 11 breakpoints with balanced/unbalanced rearrangements, complex karyotypes, primary or secondary types of AML or with adult/childhood leukemia

- to assess the potential relationship between identified chromosome 11 breakpoints and clinical parameters and to assign prognostic significance of these changes

- to establish the importance of molecular-cytogenetic screening focused on the most common chromosomal changes in AML (11q23.3 abnormalities, deletion of the 5q and 7q, monosomy 7 and numerical changes of chromosomes 8 and 9)

3. Materials and methods

Patients: We examined 300 newly diagnosed adult patients (159 males/141 females with a median age of 55 years and a range from 19 to 84 years) and 14 childhood patients (7 girls/7 boys, median age of 6.4 years, range from 1 to 15 years) with AML (243 primary AML including all children, 71 secondary AML). These patients were assessed according to the French-American-British (FAB) and/or World Health Organization (WHO) classifications. FAB subtypes were as follows: M2 (n=84), M1 (n=59), M4 (n=57), M5 (n=33), M0 (n=16), M6 (n=12), M7 (n=1) and non-specified (n=38) in adults and M5 (n=5), M4 (n=4), M2 (n=2), M0 (n=2) and M7 (n=1) in children. All patients provided written informed consent.

Conventional cytogenetics: At diagnosis, cytogenetic studies were performed on bone marrow or peripheral blood cells (if blasts were presented), using standard G-banding techniques (Wright staining).

Fluorescence in situ hybridization (FISH) analyses: The complex aberrations were investigated by multicolor FISH (mFISH) and multicolor banding (mBAND). All patient samples were examined by FISH, using the Vysis LSI MLL Break Apart Rearrangement probe (11q23.3) (Abbott). The chromosome 11 breakpoints were investigated by bacterial artificial chromosome (BAC) probes (BlueGnome). In 245 patients, the FISH screening for the most common chromosome abnormalities in AML was applied: del(7)(q31)/-7 (LSI 7q31/CEP 7 probe), del(5)(q31) (LSI 5q31/5p15.2 probe) and +8 (CEP 8/control – usually CEP 9).

Microarray methods: For the identification of chromosome 11 and genomic imbalances in general, a SNP arrays (HumanCytoSNP-12 BeadChips, Illumina) were used in ten cases.

All methods were prepared according to the manufacturer´s recommended protocols.

Statistic analysis: Differences of patient survival time were examined by Kaplan-Mayer analysis with Mantel-Cox test.

4. Results

4.1. Chromosome 11 abnormalities in adults with AML

Chromosome 11 abnormalities were confirmed in 54 (18%) of the 300 adult patients with newly diagnosed AML. Five patients had trisomy 11 and the absence of chromosome 11 breakpoints. In eleven patients, more than one chromosome 11 breakpoint was detected in the same homologue; there were two breakpoints in four cases and three or more breakpoints in seven cases. In two patients, both homologues of chromosome 11 were affected.

Aberrations in the short arm of chromosome 11 (11p) were confirmed in thirteen patients. Additional chromosomal changes were detected in all cases. Aberrations in the long arm of chromosome 11 (11q) were revealed in 40 patients. Four of them had abnormalities in 11p as well. In 17 patients, 11q alterations were detected with more than one numerical abnormality or with other structural aberrations.

Chromosome 11 breakpoint mapping

Using conventional cytogenetics, mFISH and/or mBAND, twelve different breakpoints were revealed: 11p15 (n=7), 11p14 (n=2), 11p13 (n=6), 11p12 (n=1), 11p11.2 (n=6), 11p11.1 (n=2), 11q12 (n=2), 11q13 (n=3), 11q14-q21 (n=3), 11q22 (n=3), 11q23.3 (n=30) and 11q24 (n=3). The most frequently altered regions were mapped by FISH with BAC probes and with SNP microarray in ten patients. Two patients were excluded from the gene mapping because no material was available. By SNP array, new cryptic deletions were revealed in four patients. In four cases, the breakpoint was localized to another chromosomal band/subband. FISH analysis identified new chromosome 11 aberrations and breakpoints in two cases. Using FISH mapping, localizations of the mBAND chromosome 11 breakpoints were changed in nine cases; the changes were primarily to neighboring bands or subbands. In one patient, the cryptic deletion detected by SNP microarray was not confirmed by FISH. Using a combination of FISH and SNP microarray, we identified at least 36 different chromosome 11 breakpoints (see Table 1).

Comparisons of clinical and cytogenetic data of identified chromosome 11 breakpoints are summarized in Table 2. and 3.

Table 1. Identified chromosome 11 breakpoints in AML and the candidate genes

Region	Frequency	Localization (Mb)	Candidate genes
11p15.5	5	ch11:0-3.52	cca 50 genes (HRAS, CD151, MUC6, MUC2, CARS)
11p15.4	3	ch11:3.73-3.81	NUP98
11p15.4-p15.1	3	ch11:3.87-27.92	cca 80 genes (RRM1, EIF3F, LMO1, HTATIP2, FANCF)
11p14.1	1	ch11:28.56-29.15	lincRNA
11p13	1	not mapped	
	1	ch11:29.31-31.06	FSHB, KCNA4, C11orf46, MPPED2, DCDC5, DCDC1
	1	ch11:30.98-31.06	DCDC5, DCDC1
	2	ch11:30.98-31.80	DCDC5, DCDC1, DNAJC24, ELP4, IMMP1L, miRNA
	1	ch11:31.06-31.64	DNAJC24, ELP4, IMMP1L, miRNA
	1	ch11:36.42-36.75	TRAF6, RAG1, RAG2, C11orf74
11p12	1	ch11:36.75-37.06	No protein/RNA coding gene
	1	ch11:36.88-37.49	No protein/RNA coding gene
	1	ch11:37.65-40.03	RNA genes: SNORA31, U6, lincRNA
	1	ch11:40.18-40.26	LRRC4C
	1	ch11:41.87-42.06	lincRNA
	1	ch11:42.06-43.06	lincRNA
	1	ch11:42.90-43.29	No protein/RNA coding gene
11p11.2	1	not mapped	
	1	ch11:44.48-45.08	CD82, TP53I11, TSPAN18,lincRNA
	1	ch11:46.37-46.54	DGKZ, MDK, CHRM4, AMBRA1
	1	ch11:47.13-47.23	ARFGAP2, PACSIN3
	1	ch11:47.33-47.39	SPI1, MYBPC3, MADD
11p11.1	1	not mapped	
11q12	2	not mapped	
11q13.2	1	ch11:67.42-67.90	NDUFS8, UNC93B1, ALDH3B1, TCIRG1, CHKA,
	1	ch11:67.64-68.31	ALDH3B2,lincRNA, miRNA, U6 RNA
	2	ch11:68.31-68.52	GAL, MTL5, lincRNA
11q14.1	1	ch11:78.39-78.52	ODZ4
11q14.2	1	ch11:79.11-87.47	cca 25 genes (ODZ4, RSF1, THRSP, GAB2, PICALM,
	1	ch11:79.11-92.28	MIR708, RAB30, SYTL2, EED, FZD4)
	1	ch11:87.65-92.10	cca 20 genes (RAB38, TYR, NOX4, TRIM49, NAALAD2, CHORDC1, FAT3)
	1	ch11:92.10-94.62	cca 30 genes (FAT3, MRE11A, MTNRIB, PANX1, FUT4)
11q21	1	ch11:94.62-95.34	KDM4D, ENDOD1, CWC15, SESN3, KDM4DL
	1	ch11:95.16-95.64	CEP57, FAM76B, MTMR2, 5S RNA, lincRNA
	1	ch11:95.85-104.45	cca 30 genes (MAML2)
	1	ch11:95.85-96.07	MAML2
11q22.1	1	not mapped	
11q22.3	1	ch11:105.51-106.38	GRIA4, U6, U4, KIAA1826, KBTBD3, AASDHPPT,
	1	ch11:106.54-108.09	lincRNA
			cca 11 genes (GUCY1A2, ALKBHB, RAB39A, CUL5, ACAT1, NPAT)
11q23.1	1	ch11:109.76-110.70	ARGHAP20, RDX, ZC3H12C, FDX1
11q23.3	1	ch11:110.70-113.60	cca 30 genes (POU2AF1, MIR34B, MIR34C, CRYAB, SIK2)
	1	ch11:113.79-118.00	cca 30 genes (ZBTB16, NNMT, TAGLN, APOA4, APOC3)
	1	ch11:118.00-118.30	cca 10 genes (TMPRSS4, UBE4A, AMICA1, MPZL2)
	28	ch11:118.30-118.39	MLL
11q24	1	not mapped	
	1	ch11:121.00-121.50	TECTA, SC5DL, SORL1
	1	ch11:124.50-125.00	cca 25 genes (SPA17, ESAM, ROBO3, ROBO4, HEPACAM)
11q25	1	ch11:131.50-132.50	OPCML, NTM, C11orf39

Abbreviations: recurrent breakpoints, possibly non-random breakpoints, overlapping regions.

Table 2. Comparison of clinical and cytogenetic data of 54 AML patients according to the affected chromosome 11 arm.

Affected arm	Recurrent breakpoint	Count*	Ø age	F/M	P/S	FAB type (n)	Mechanism of breakpoint (n)	Complex karyotype	1. remission	aBMT	living	Ø survival (months)
11p	11p15.4 (*NUP98*) 11p13 11p12	13/24% (4x with 11q)	61	5/8	9/4	M2 (4), M4 (4), M0 (2), M1(1), M6 (1),other (1)	BR (6) Deletion (6) Dupl/Amp (5)	13	3	0	2	4
11q (*MLL* gene)	11q23.3 (*MLL*)	28/52% (1x with 11p)	46	15/13	20/8	M5 (14), M1(7), M4 (4), M2 (2), other (1)	BR (25) UR (3)	3	15	12	10	4
11q (others)	11q13.2	14/26% (3x with 11p)	62	6/8	10/4	M2(4), M4 (3), M0 (2), M5 (2), other (3)	BR (4) Dupl/Amp(10) Deletion (3)	10	6	2	2 (1 with *MLL* alteration)	4

Abbreviations: * number/% out of patients with chromosome 11 abnormalities; F, female; M, male, P, primary; S, secondary; BR, balanced rearrangement; UR, unbalanced rearrangement; n, count; Dupl/Amp, duplication/amplification; aBMT, allogeneic bone marrow transplantation.

Table 3. Comparison of clinical and cytogenetic data of 54 AML patients according to the recurrent chromosome 11 breakpoint.

Region	Localization (Mb)	Genes	Conunt*	Ø age	F/M	P/S	FAB type (n)	Mechanism of breakpoint (n)	Complex karyotype	1. remission	aBMT	living	Ø survival (months)
11p15.4	ch11:3.73-3.81	*NUP98*	3/6%	58	1/2	3/0	M2 (2), M4 (1)	BR (2) Duplication (1)	3	2	0	0	8
11p13	ch11:30.98-31.06	*DCDC5*, *DCDC1*	4/7%	64	1/3	4/0	M4 (2), M0 (1) M2 (1)	BR (1) UR (2) Deletion (1)	4	1	0	0	15
11p12	ch11:36.75-37.49	no	2/4%	71	1/1	2/0	M0 (1), M1(1)	BR (1) Deletion (1)	2	0	0	0	2
11q13.2	ch11:68.31-68.52	*GAL*, *MTL5*, lincRNA	2/4%	54	0/2	2/0	M2 (1), M5 (1)	BR (2)	1	2	1	1	22
11q23.3	ch11:118.30-118.39	*MLL*	28/52%	46	15/13	20/8	M5(14), M1(7) M4 (4), M2 (2) other (1)	BR (25) UR (3)	3	15	12	10	15

Abbreviations: * number/% out of patients with chromosome 11 abnormalities; F, female; M, male, P, primary; S, secondary; BR, balanced rearrangement; UR, unbalanced rearrangement; n, count; aBMT, allogeneic bone marrow transplantation.

Areas of amplification/duplication and deletion

In 25 out of 35 (71%) patients with balanced chromosome 11 rearrangements, the *MLL* gene was affected.

The gain of chromosome 11 involved 11q in all cases. Partial trisomy was observed in eight cases. The common area of duplication was 11q23.3→11q24 (ch11:118.35-125.00 Mb), starting from the 3′ end of *MLL*.

Chromosome 11 amplification (more than three copies of the region) was observed in nine patients. One patient had an amplification only in 11p. The commonly amplified region of 11q included the 5′ end of *MLL* (n=8).

Partial loss of chromosome 11 was observed in nine patients. In only three cases, the 11q area was involved. We did not confirm one common deleted region. However, two regions were lost more often: 11p13 (n=4; ch11:31.00-31.50 Mb), 11p14.1→11p13 (n=4; ch11:28.00-31.00 Mb), and 11pter-11p15.5 (n=4; ch11:0-3.52 Mb).

Partial tandem (PTD) and nontandem (PNTD) *MLL* duplication

The *MLL* PTD was proved in 50 of 210 (23%) newly diagnosed adult AML patients (23 female/27male, average age 55 years, 9x secondary AML). The patients were diagnosed with AML: M2 (n=18), M1 (n=13), M4 (n=10), M0 (n=4), M5 (n=2), M6 (n=2), other (n=1). *MLL* PTD were detected with: normal karyotype (n=21), 0 mitosis (n=3), t(8;21)/inv(16) (n=4), complex karyotype (n=5, 2x chromosome 11 aberration and 1x trisomy 11), *MLL* gene rearrangement (n=7), and with other chromosome changes (n=10, 1x partial trisomy 11, 1x trisomy 11, 2x monosomy 7). Duplication of the exones 2 to 6 was the most frequent. The *MLL* PNTD was proved in 3 females with t(9;11)(p22;q23), t(11;19)(p13;q23) and t(9;12;11)(p22;p13;q23).

4.2. Chromosome 11 abnormalities in children with AML

Chromosome 11 abnormality was proved in five out of 14 children with newly diagnosed AML (36%). In all cases (3 girls/2 boys, average age 2 years, de novo AML M5), a balanced *MLL* gene rearrangement was detected. All patients are alive, however the observation time of survival have been too short to define the prognostic impact (<1 year in four of them).

5. Discussion

In this study, we described chromosome 11 abnormalities in 18% of newly diagnosed adult patients with AML. The aberrations affected the 11q region more often than 11p. The alterations of 11p were always part of a complex karyotype. The instability of chromosome 11 in AML was confirmed by the presence of more than two chromosome 11 breakpoints detected in eleven patients and breakpoints that affected both chromosome 11 homologues in two cases.

Using a combination of FISH and SNP microarrays, at least 36 different breakpoints on chromosome 11 were identified. In agreement with the literature, the most frequent breakpoint of chromosome 11 was located in the *MLL* gene (11q23.3) (Meyer et al. 2009). In seven patients, only FISH was able to detect *MLL* gene rearrangements, primarily due to its cryptic character. Park et al. (2008) confirmed that some *MLL* translocations escape detection when routine cytogenetic methods are the only diagnostic test used in the clinical laboratory. The most common translocations in our cohort of patients were t(9;11)(p22;q23) (n=11) and t(11;19)(q23;p13.3) (n=4). However, we found two three-way translocations, and we were the first to identify a new reciprocal *MLL* translocation of t(6;11)(p21.3;q23). In three cases, we detected a very rare *MLL* gene abnormality - a partial nontadem *MLL* gene duplication (*MLL* PNTD). The *MLL* PNTD is generated as a consequence of multiple recombination events, however the mechanisms triggering the formation of *MLL* PNTD remain unclear (Whitman et al. 2005, Sarova et al. 2009).

In three patients, the breakpoint was identified in the *NUP98* gene (11p15.4). In two cases, a balanced variant of translocations t(5;11;20)(q35;p15;p?) and t(4;5;11)(q11;q35;p15) were observed. The t(5;11) translocation usually has a cryptic character; it has been detected with an apparently normal karyotype or with deletion del(5q) (Jaju et al. 2001). In one of our cases, we did not detect a *NUP98* rearrangement by mFISH/mBAND or microarray.

In this report, three possibly nonrandom breakpoints were described in the 11p13, 11p12 and 11q13.3 bands. Three overlapping regions were mapped in 11p13: ch11:30.98-31.06 Mb, ch11:29.31-31.06 Mb, and ch11:30.98-31.80 Mb. The first of these regions spans only the *DCDC5* and *DCDC1* genes. The second and third regions involve the aforementioned genes and a few others. Thus, *DCDC5* and *DCDC1*

appear to be important candidates for genes affected in these rearrangements. Both genes play an important role in microtubule polymerization, which is necessary for cell division and cytoplasmic transport. In the 11p12 (ch11:36.75-37.49 Mb) region, no protein or RNA-coding genes have been observed or predicted. The two candidate genes are located in the 11q13.3 breakpoint (ch11:68.31-68.52 Mb): *GAL* and *MTL5*. Galanin is a neuropeptide and thus acts as a transfer molecule between the central and peripheral nervous systems. Its deregulation has already been demonstrated in some solid tumours (Rauch and Kofler, 2010). The *MTL5* gene plays a role in spermatogenesis, cell growth and differentiation.

Overlapping regions were also detected in chromosomal bands 11p15.5 (ch11:0-3.52 Mb), 11p15.4-p15.1 (ch11:3.87-27.92 Mb), 11p12 (42.06-43.29 Mb), 11q12 (not mapped), 11q13.2 (67.42-68.31 Mb), 11q14.2-q21 (79.11-104.45 Mb) and 11q24 (not mapped). Due to a shortage of bone marrow cells, these regions were not as precisely defined as the others. Therefore, it is not appropriate to classify them as a truly recurrent.

Five breakpoint sites involved only one protein coding gene: *NUP98* (11p15.4), *MLL* (11q23.3), *LRRC4* (11p12), *ODZ4* (11q14.1) or *MAML2* (11q21). The breakpoint in the *LRRC4* and the *ODZ4* was detected in only one patient. It is possible that these breakpoints are random and play no significant role in leukemogenesis. Two overlapping breakage sites were observed at 11q21: ch11:95.85-96.07 Mb that involved only the *MAML2* gene and ch11:95.85-104.45 Mb, which covers approximately 30 genes and includes *MAML2*. The *MAML2* gene is a transcriptional co-activator for the NOTCH receptors, participating in signal transduction and resulting in the activation of NOTCH downstream targets. As *MLL* fusion partner gene, *MAML2* has been reported in AML and myelodysplastic syndromes (Nemoto et al. 2007).

Many chromosome 11 breakpoints were generated by unbalanced chromosome 11 changes. Deletions involved mainly 11p areas. We did not identify a single common deleted region. Nevertheless, some areas were affected more often than others, such as 11pter-11p15.5 (n=4; ch11:0-3.52 Mb), 11p14.1-11p13 (n=4; ch11:28.00-31.00 Mb), and 11p13 (n=4; ch11:31.00-31.50 Mb). There are a lot of known and predicted tumor suppressor genes located at 11p15.5, such as *MIR210* (microRNA suppressing cancer cell survival and proliferation), *MUC6*, *MUC2*,

MUC5B (mucins with roles in proliferation and metastases), *CDKN1C* (cyclin dependent kinas inhibitor, p57) or *RPLP2* (ribosomal protein). In the 11p14.1-p13 and 11p13 region, only a few protein-coding genes are presented: *MPPED2* (an inhibitor of proliferation), *DCDC5*, *DCDC1* (involved in the polymerization of microtubulin), *DNAJC24* (a stimulator of ATPases), and *IMMPL* (a mitochondrial peptidase).

We proved that duplication/amplification of the chromosome 11 is nonrandom recurrent aberration in AML (Sarova et al. 2010). The gain of chromosome 11 genetic material involved the 11q in all cases. Partial trisomy 11 was observed in eight patients. The common area of duplication was 11q23.3→11q24 (ch11:118.35-125.00 Mb), starting from the 3´ end of *MLL*. Amplification (more than three copies) was confirmed in nine patients. One patient had an amplification of only the 11p area. The common region of 11q amplification was the 5´end of *MLL*. The second most common amplified region was 11q23.3→11q25 (ch11:118.00-132.50 Mb). Using a CGH array, Rucker et al. (2006) identified three common amplified 11q regions: 11q12-q14, 11q23.3 and 11q23.3-q24.1. Zatkova et al. (2009) detected three amplified areas in all of the investigated patients: 11q23.3, 11q24.2-q24.3 and 11q24.3-q25. We can summarize that the chromosome 11 amplicons vary in size however usually include the *MLL* gene, parts of the gene and distal chromosomal bands of 11q23.3-q25 (Sarova et al. 2010).

There are a lot important genes in the 11q23.3→11q25 region as follows: *MLL* (transcription factors), *DDX6* (RNA helicase), *ARHGEF12* and *SPA17* (signal transduction), *H2AFX* (histon protein), *ETS1* (apoptosis inhibitor), *UBE4A* (ubiquitination pathway) and *miR-100* (miRNA). Many other studies have confirmed the involvement of the *MLL* and *DDX6* gene amplification in AML (Rucker et al. 2006, Zatkova et al. 2009). MicroRNA 100 is a member of small non-coding RNAs of approximately ~ 22 nucleotides in length, play important roles in the differentiation of various cell types and in the initiation and progression of cancer. Wooi Loon Ng et al. (2010) showed that the over-expression of miR-100 is responsible for the low-expression of the tumour suppressor gene *ATM* (11q22). ATM is one of the most important checkpoint proteins in mammalian cells. The gene promotes homologous recombination repair, but it is also involved in non-homologous end joining.

We proved that the FISH mapping with BAC probes is an essential tool in cancer research and identification of altered genes, however there are some limitations to this

approach, including the large inserts and requiring a large amount of material. Therefore, the use of microarrays for unbalanced changes is recommended (Bystricka et al. 2010, De Braekeleer et al. 2011). Also we confirmed the suggestion by identification of new cryptic deletions in our cohort of AML patients.

From the clinical point of view, we showed that the chromosome 11 changes is an important prognostic marker. The patients with chromosome 11 abnormalities can be stratified into two relatively homogenous groups: patients with *MLL* rearrangements and patients with other chromosome 11 changes. We confirmed that patients with *MLL* gene rearrangement are usually young people (<50 years) diagnosed as AML M5, M1 or M4 and characterized by balanced aberrations. Because of ordinary indication of these patients for allogeneic bone marrow transplantation, the average survival was approximately 15 months. On the other hand, patients with other chromosome 11 changes regardless of breakpoint localization were mostly older (>60 years) with complex karyotype, unbalanced aberrations (deletions of 11p and gain of 11q) and very poor clinical outcome (average survival only 4 months). The most observed FAB subtype of AML patients with other chromosome 11 abnormality were M2 and M4, generally the most frequent FAB subtypes in AML.

In childhood cohort of AML patients, we proved higher frequency (36 %) of chromosome 11 abnormalities than in adults. In all cases, the *MLL* gene was affected by balanced aberration. Children were usually 1 year olds and always diagnosed with AML M5. Because of the short observation time (<1 year in four of them), we are not able to define the true prognostic impact.

Due to the cryptic character of many genetic abnormalities, we also suggest the probe panel for the most frequent chromosome aberrations (including del(5q), del(7q)/-7, *MLL* gene rearrangements and +8) under the FISH screening applied in AML patients at the time of diagnosis. We proved the significant importance of the FISH screening, especially, for the *MLL* gene aberrations which are often cryptic and under the resolution of conventional cytogenetics. FISH screening was also very beneficial in cases with conventional examination failure.

6. Conclusion

Nowadays, conventional cytogenetics is important diagnostic and prognostic tool in patients with leukemia. Therefore, it belongs to the standard investigation methods in these patients. However, there are some limits such as cells proliferation, chromosomal morphology, extent of pathological clone with chromosomal aberration and resolution. In these cases, FISH analysis is a suitable informative supplement as same as in patients where are not present any mitoses for karyotyping. Conventional and molecular cytogenetic analyses are also essential for identification of affected chromosome regions and genes in leukemia and for clarification of malignant transformation processes.

In this study, we identified and defined the chromosome 11 breakpoints and common deleted and amplified areas in the bone marrow or peripheral blood cells of newly diagnosed patients with AML by molecular cytogenetic methods. Between years 2006 to 2012, we examined 300 adult and 14 childhood AML patients in the Cytogenetic department of IHBT and CNC VFN. The chromosome 11 change was proved in bone marrow/peripheral blood cells of 18% of adult patients and of 36% of childhood patients with AML. The results are summarized below.

- Chromosome 11 abnormalities were proved as a frequent chromosomal change found in adult newly diagnosed AML patients. The instability of chromosome 11 in AML was confirmed by the presence of more than two chromosome 11 breakpoints and by breakpoints affecting both chromosome 11 homologues.

- Many recurrent and random chromosome 11 breakpoints were identified (recurrent in the *NUP98* gene (11p15.4) and *MLL* (11q23.3), and three possibly nonrandom in chromosomal bands 11p13, 11p12 and 11q13.2). New candidate genes that are possibly significant in the origin and/or progress of AML were notified.

- The most common deleted, duplicated/amplified regions and candidate tumor suppressor genes and oncogenes were determined. The potential involvement and contribution of affected areas of chromosome 11 to the malignant transformation process was discussed.

- The chromosome 11 changes were assessed as an important prognostic marker. From the clinical point of view, the chromosome 11 abnormalities can be stratified into two relatively homogenous groups:
    a) patients with *MLL* rearrangements
    b) patients with other chromosome 11 changes.

- The *MLL* gene rearrangements were mostly detected in young people (<50 years) diagnosed as AML M5, M1 or M4 and with balanced aberrations. The average survival was approximately 15 months.

- Patients with other chromosome 11 changes regardless of breakpoint localization were mostly older (>60 years) with complex karyotype, unbalanced aberrations (deletions of 11p and gain of 11q) and very poor clinical outcome (average survival only 4 months). No significant relationship between chromosome 11 breakpoints and types of AML were observed.

- Higher frequency of chromosome 11 abnormalities in children than in adults with AML was proved. In all cases, *MLL* gene was affected by balanced aberration. Children were younger (about 1 year) and always diagnosed with FAB subtype M5.

- FISH screening as a rapid detection of pathological clones with unfavourable prognosis was proved very helpful in case of dividing cells lack and cryptic *MLL* gene rearrangement.

In conclusion, molecular analyses of chromosomal breakpoints and amplified or deleted areas are very important for the identification of genes involved in tumour pathogenesis and progression. Further investigation of the affected genes and their protein products will improve our understanding of malignant transformation, basic mechanisms of chromosomal and molecular rearrangements and their role in oncogenesis. In addition, obtained knowledge could be clinically applied during diagnosis, allowing patient stratification into prognostic subgroups, facilitating residual disease monitoring and could be also used for the designation of more effective therapeutic approach.

7. References

1. Babicka L, Ransdorfova S, Brezinova J, Zemanova Z, Sindelarova L, Siskova M, Maaloufova J, Cermak J, Michalova K (2007): Analysis of complex chromosomal rearrangements in adult patients with MDS and AML by multicolor FISH. Leukemia Research, 31(1):39-47.

2. Balgobind BV, Raimondi SC, Harbott J, Zimmermann M, Alonzo TA, Auvrignon A, Beverloo HB, Chang M, Creutzig U, Dworzak MN, Forestier E, Gibson B, Hasle H, Harrison CJ, Heerema NA, Kaspers GJ, Leszl A, Litvinko N, Nigro LL, Morimoto A, Perot C, Pieters R, Reinhardt D, Rubnitz JE, Smith FO, Stary J, Stasevich I, Strehl S, Taga T, Tomizawa D, Webb D, Zemanova Z, Zwaan CM, van den Heuvel-Eibrink MM (2009): Novel prognostic subgroups in childhood 11q23/MLL-rearranged acute myeloid leukemia: results of an international retrospective study. Blood, 114(12):2489-96.

3. Basecke J, Podleschny M, Clemens R, Schnittger S, Viereck V, Trumper L, Griesinger F (2006): Lifelong persistence of AML associated MLL partial tandem duplications (MLL-PTD) in healthy adults. Leuk Res, 30(9):1091-1096.

4. Bystřická D, Zemanová Z, Březinová J, Gančarčíková M, Grosová L, Šárová I, Izáková S, Berková A, Michalová K (2010): The assessment of array comparative genomic hybridization in complex karyotype analyses. Folia Biol (Praha), 56(5):223-30.

5. Casas S, Aventín A, Nomdedéu J, Sierra J (2003): Cryptic t(5;11)(q35;p15.5) in adult de novo acute myelocytic leukemia with normal karyotype. Cancer Genet Cytogenet, 145(2):183.

6. De Braekeleer E, Douet-Guilbert N, Basinko A, Morel F, Le Bris MJ, Férec C, De Braekeleer M (2011): Using bacterial artificial chromosomes in leukemia research: the experience at the university cytogenetics laboratory in Brest, France. J Biomed Biotechnol 329471.

7. Hanahan D, Weinberg RA (2011): Hallmarks of cancer: the next generation. Cell, 144(5):646-74.

8. Hollink IH, van den Heuvel-Eibrink MM, Arentsen-Peters ST, Pratcorona M, Abbas S, Kuipers JE, van Galen JF, Beverloo HB, Sonneveld E, Kaspers GJ, Trka J, Baruchel A, Zimmermann M, Creutzig U, Reinhardt D, Pieters R, Valk PJ, Zwaan CM (2011): NUP98/NSD1 characterizes a novel poor prognostic group in acute myeloid leukemia with a distinct HOX gene expression pattern. Blood, 29;118(13):3645-56.

9. Cho WC (2007): OncomiRs: the discovery and progress of microRNAs in cancers. Mol Cancer, 6:60.

10. Iwamoto M, Asakawa H, Hiraoka Y, Haraguchi T (2010): Nucleoporin Nup98: a gatekeeper in the eukaryotic kingdoms. Genes Cells, 15(7):661-9.

11. Jaju RJ, Fidler C, Haas OA, Strickson AJ, Watkins F, Clark K, Cross NC, Cheng JF, Aplan PD, Kearney L, Boultwood J, Wainscoat JS (2001): A novel gene, NSD1, is fused to NUP98 in the t(5;11)(q35;p15.5) in de novo childhood acute myeloid leukemia. Blood, 98(4):1264-7.

12. La Starza R, Brandimarte L, Pierini V, Nofrini V, Gorello P, Crescenzi B, Berchicci L, Matteucci C, Romoli S, Beacci D, Rosati R, Martelli MF, Mecucci C (2009): A NUP98-positive acute myeloid leukemia with a t(11;12)(p15;q13) without HOXC cluster gene involvement. Cancer Genet Cytogenet, 193(2):109-11.

13. Marchesi F, Annibali O, Cerchiara E, Tirindelli MC, Avvisati G (2011): Cytogenetic abnormalities in adult non-promyelocytic acute myeloid leukemia: a concise review. Crit Rev Oncol Hematol, 80(3):331-46.

14. Meyer C, Kowarz E, Hofmann J, Renneville A, Zuna J, Trka J, Ben Abdelali R, Macintyre E, De Braekeleer E, De Braekeleer M, Delabesse E, de Oliveira MP, Cavé H, Clappier E, van Dongen JJ, Balgobind BV, van den Heuvel-Eibrink MM, Beverloo HB, Panzer-Grümayer R, Teigler-Schlegel A, Harbott J, Kjeldsen E, Schnittger S, Koehl U, Gruhn B, Heidenreich O, Chan LC, Yip SF, Krzywinski M, Eckert C, Möricke A, Schrappe M, Alonso CN, Schäfer BW, Krauter J, Lee DA, Zur Stadt U, Te Kronnie G, Sutton R, Izraeli S, Trakhtenbrot L, Lo Nigro L, Tsaur G, Fechina L, Szczepanski T, Strehl S, Ilencikova D, Molkentin M, Burmeister T, Dingermann T, Klingebiel T, Marschalek R (2009): New insights to the MLL recombinome of acute leukemias. Leukemia, 23(8):1490-9.

15. Mitelman F (2000): Recurrent chromosome aberrations in cancer. Mutat Res, 462(2-3):247-53.

16. Mrózek K (2008): Cytogenetic, molecular genetic, and clinical characteristics of acute myeloid leukemia with a complex karyotype. Semin Oncol, 35(4):365-77.

17. Mrózek K, Heerema NA, Bloomfield CD (2004): Cytogenetics in acute leukemia. Blood Rev, 18(2):115-36.

18. Mrózek K, Marcucci G, Paschka P, Whitman SP, Bloomfield CD (2007): Clinical relevance of mutations and gene-expression changes in adult acute myeloid leukemia with normal cytogenetics: are we ready for a prognostically prioritized molecular classification? Blood, 109(2):431-48.

19. Nemoto N, Suzukawa K, Shimizu S, Shinagawa A, Takei N, Taki T, Hayashi Y, Kojima H, Kawakami Y, Nagasawa T (2007): Identification of a novel fusion gene MLL-MAML2 in secondary acute myelogenous leukemia and myelodysplastic syndrome with inv(11)(q21q23). Genes Chromosomes Cancer, 46(9):813-9.

20. Ng WL, Yan D, Zhang X, Mo YY, Wang Y (2010): Over-expression of miR-100 is responsible for the low-expression of ATM in the human glioma cell line: M059J. DNA Repair (Amst), 9(11):1170-5.

21. Park TS, Lee ST, Song J, Lee KA, Lee SG, Kim J, Suh B, Kim SJ, Lee JH, Park R, Choi JR (2008): MLL rearrangement with t(6;11)(q15;q23) as a sole abnormality in a patient with de novo acute myeloid leukemia: conventional cytogenetics, FISH, and multicolor FISH analyses for detection of rare MLL-related chromosome abnormalities. Cancer Genet Cytogenet, 187(1):50-3.

22. Rauch I, Kofler B (2010): The galanin system in cancer. EXS 102:223-41.

23. Rücker FG, Bullinger L, Schwaenen C, Lipka DB, Wessendorf S, Fröhling S, Bentz M, Miller S, Scholl C, Schlenk RF, Radlwimmer B, Kestler HA, Pollack JR, Lichter P, Döhner K, Döhner H (2006): Disclosure of candidate genes in acute myeloid leukemia with complex karyotypes using microarray-based molecular characterization. J Clin Oncol, 24(24):3887-94.

24. Sandberg AA, Meloni-Ehrig AM (2010): Cytogenetics and genetics of human cancer: methods and accomplishments. Cancer Genet Cytogenet, 203(2):102 - 26.

25. Sárová I, Brezinová J, Zemanová Z, Izáková S, Lizcová L, Malinová E, Berková A, Cermák J, Maaloufová J, Nováková L, Michalová K (2010): Cytogenetic manifestation of chromosome 11 duplication/amplification in acute myeloid leukemia. Cancer Genet Cytogenet, 199(2):121-7.

26. Sárová I, Brezinová J, Zemanová Z, Lizcová L, Berková A, Izáková S, Malinová E, Fuchs O, Kostecka A, Provazníková D, Filkuková J, Maaloufová J, Starý J, Michalová K (2009): A partial nontandem duplication of the MLL gene in four patients with acute myeloid leukemia. Cancer Genet Cytogenet, 195(2):150-6.

27. Taylor TD, Noguchi H, Totoki Y, Toyoda A, Kuroki Y, Dewar K, Lloyd C, Itoh T, Takeda T, Kim DW, She X, Barlow KF, Bloom T, Bruford E, Chang JL, Cuomo CA, Eichler E, FitzGerald MG, Jaffe DB, LaButti K, Nicol R, Park HS, Seaman C, Sougnez C, Yang X, Zimmer AR, Zody MC, Birren BW, Nusbaum C, Fujiyama A, Hattori M, Rogers J, Lander ES, Sakaki Y (2006): Human chromosome 11 DNA sequence and analysis including novel gene identification. Nature, 440(7083):497-500.

28. Wang Y, Xue Y, Chen S, Wu Y, Pan J, Zhang J, Shen J (2010): A novel t(5;11)(q31;p15) involving the NUP98 gene on 11p15 is associated with a loss of the EGR1 gene on 5q31 in a patient with acute myeloid leukemia. Cancer Genet Cytogenet, 199(1):9-14.

29. Whitman SP, Liu S, Vukosavljevic T, Rush LJ, Yu L, Liu Ch, Klisovic MI, Maharry K, Guimond M, Strout MP, Becknell B, Dorrance A, Klisovic RB, Plass Ch, Bloomfield CD, Marcucci G, Caligiuri MA (2005): The MLL tandem duplication: evidence for recessive gain-of-function in acute myeloid leukemia identifies a novel patient subgroup for molecular-targeted therapy. Blood, 1:345-352.

30. Whitman SP, Strout MP, Marcucci G, Freud AG, Culley LL, Zeleznik-Le NJ, Mrózek K, Theil KS, Kees UR, Bloomfield CD, Caligiuri MA (2001): The partial nontandem duplication of the MLL (ALL1) gene is a novel rearrangement that generates three distinct fusion transcripts in B-cell acute lymphoblastic leukemia. Cancer Res, 61(1):59-63.

31. Zatkova A, Merk S, Wendehack M, Bilban M, Muzik EM, Muradyan A, Haferlach C, Haferlach T, Wimmer K, Fonatsch C, Ullmann R (2009): AML/MDS with 11q/MLL amplification show characteristic gene expression signature and interplay of DNA copy number changes. Genes Chromosomes Cancer 48(6):510-20.

8. List of publications

8.1. Publications with relation to PhD thesis

Publications

Šárová I, *Březinová J, Zemanová Z, Lizcová L, Berková A, Izáková S, Malinová E, Fuchs O, Kostečka A, Provazníková D, Filkuková J, Maaloufová J, Starý J and Michalová K:* A partial nontandem duplication of the MLL gene in four patients with acute myeloid leukemia. Cancer Genetics and Cytogenetics 2009, 195:150-156. **IF09 1.537**

Sárová I, *Brezinová J, Zemanová Z, Izáková S, Lizcová L, Malinová E, Berková A, Cermák J, Maaloufová J, Nováková L, Michalová K:* Cytogenetic manifestation of chromosome 11 duplication/amplification in acute myeloid leukemia. Cancer Genet Cytogenet. 2010 Jun;199(2):121-7. **IF10 1.551**

Bystřická D, Zemanová Z, Březinová J, Gančarčíková M, Grosová L, ***Šárová I,*** *Izáková S, Berková A, Michalová K:* The assessment of array comparative genomic hybridization in complex karyotype analyses. Folia Biol (Praha). 2010;56(5):223-30. **IF10 0.729**

Sárová I, *Březinová J, Zemanová Z, Gančarčíková M, Vydra J, Cermák J, Michalová K:* A novel gene LRP5 on 11q13.2 is rearranged in two patients with acute myeloid leukemia. Leuk Res. 2011 Nov;35(11):e200-2. **IF11 2.923**

Sarova I*, Brezinova J, Zemanova Z, Bystricka D, Krejcik Z, Soukup P, Cetkovský P, Vydra J, Cermak J, Jonasova A and Michalova K:* Characterization of chromosome 11 breakpoints and the areas of deletions and amplifications in newly diagnosed acute myeloid leukemia.
Odesláno k publikaci v Genes, chromosomes and cancer. **IF11 3.306**

Abstracts with IF

Šárová I*, Březinová J, Lizcová L, Zemanová Z, Fuchs O, Kostečka A, Provazníková D, Filkuková J, Maaloufová J and Michalová K:* The unusual MLL rearrangement in acute myeloid leukemia. 14th Congress of the European Hematology Association (Berlín, 4.-7.6.2009)
Abstrakt: Haematologica/The Hematology Journal Haematologica, 2009;94(s2):13.
IF09 6.416

Zemanova Z, Michalova K, Brezinova J, Lizcova L, Izakova S, Bystricka D, ***Sarova I****, Siskova M, Neuwirtova R, Cerna O, Cermak J:* Complex chromosomal aberrations in bone marrow cells of 86 patients with myelodysplastic syndromes (MDS). 14th Congress of the European Hematology Association (Berlín, 4.-7.6.2009)
Abstrakt: Haematologica/The Hematology Journal Haematologica, 2009;94(s2):13-14. **IF09 6.416**

Izáková S, ***Šárová I***, *Březinová J, Zemanová Z, Berková A, Lizcová L, Maaloufová J, Michalová K:* MLL amplification in patiens with acute myeloid leukemia (AML). The Europian Human Genetics (Vídeň, 23.-26.5.2009)
Abstrakt: European Journale of Human Genetics, 2009;17(2):209-210. **IF09 3.564**

Zemanova Z, Michalova K, Brezinova J, Lizcova L, Izakova S, Bystricka D, ***Sarova I****, Siskova M, Cerna O, Cermak J:* Molecular Cytogenetic Studies of Complex Chromosomal Aberrations in Myelodysplastic Syndromes (MDS). The European Cytogenetics Association (Stockholm, 4.-7.7.2009)
Abstrakt: Chromosome Research, 2009;17(1):131. **IF09 3.230**

Sarova I, *Brezinova J, Izakova S, Malinova E, Zemanova Z, Lizcova L, Cermak J, Polivka J, Vydra J, Siskova M, Michalova K:* Cytogenetic analyses of the chromosome 11 duplication/amplification in acute myeloid leukemia.
The Europian Human Genetics (Gothenburg, 12.-15.6.2010)
Abstrakt: European Journale of Human Genetics, 2010;18(1):195. **IF10 4.380**

Šárová I*, Březinová J, Bystřická D, Zemanová Z, Izáková S, Malinová E, Lizcová L, Čermák J, Jonášová A and Michalová K:* Molecular cytogenetic analysis of chromosome 11 breakpoints in acute myeloid leukemia.
8th European Cytogenetics Conference (Porto, 2.-5.7.2011)
Abstrakt: Chromosome Research 2011;19(1):S133. **IF11 3.087**

Šárová I*, Březinová J, Bystřická D, Zemanová Z, Krejčík Z, Čermák J, Vydra J, Jonášová A and Michalová K:* Chromosome 11 as a target of breakpoint mapping in acute myeloid leukemia.
The Europian Human Genetics (Norimberk, 23.-26.6.2012)
Abstrakt: European Journale of Human Genetics, 2012;20(1):202. **IF11 4.400**

Abstracts without IF

Šárová I*, Březinová J, Izáková S, Zemanová Z, Malinová E, Lizcová L, Bystřická D, Gančarčíková, Grosová L, Čermák J, Polívka J, Vydra J, Šišková M and Michalová K:* Molecular cytogenetic analysis of chromosome 11 aberrations in hematological malignancies.
International Life Science Students Conference (Nijmegen, 10.-14.11.2010)
Abstrakt: Conference book 2011:76.

Šárová I*, Březinová J, Zemanová Z, Izáková S, Maaloufová J, Čermák J, Michalová K:* Význam aberací MLL genu u nemocných s akutní myeloidní leukémií. 10. studentská vědecká konference (Praha, 27.5.2009)
Abstrakt: Sborník 10. studentské vědecké konference, 2009:54.

Šárová I*, Březinová J, Zemanová Z, Michalová K:* Mapování zlomových míst na chromosomu 11 u akutní myeloidní leukémie. 12. studentská vědecká konference (Praha, 24.5.2011)
Abstrakt: Sborník 12. studentské vědecké konference, 2011: 100-102.

***Šárová I**, Březinová J, Bystřická D, Zemanová Z, Izáková S, Malinová E, Lizcová L and Michalová K:* Molecular cytogenetic analysis of 11q13.2 breakpoints in free patiens with acute myeloid leukemia.
The Student ScientificConference on Cancer Research (Brno, 7.-8.4.2011)
Abstrakt: Conference book 2011:122.

***Šárová I**, Březinová J, Zemanová Z, Bystřická D, Čermák J, Vydra J a Michalová K:* Variantní formy kryptické translokace t(5;11)(q35;p15) u 2 nemocných s nově diagnostikovanou AML.
XVI. Česko-slovenský hematologický a transfuziologický sjezd (Brno, 5.-8.9.2012)
Abstrakt: Sborník abstrakt 2012:142.

8.2. Publications without relation to PhD thesis

Publications

*Březinová J, Berková A, Včelíková S, Zemanová Z, Izáková S, **Šárová I,** Čechová H, Tajtlová J, Grosová L, Lizcová L, Malinová E, Zemanová M, Cmunt E, Karban J, Trněný M, Schwarz J, Michalová K:* Telomere length, molecular cytogenetic findings, and immunophenotypic features in previously untreated patients with B-chronic lymphocytic leukemia. Neoplasma. 2010;57(3):215-21. **IF10 1.449**

*Brezinova J, Zemanova Z, Bystricka D, **Sarova I,** Lizcova L, Malinova E, Izákova S, Sajdova J, Sponerova D, Jonasova A, Cermak J and Michalova K:* Deletion of the long arm but not the 5q31 region of chromosome 5 in myeloid malignancies. Leuk Res. 2012 Mar;36(3):e43-5. **IF11 2.923**

Bystricka D, Sarova I, Zemanova Z, Brezinova J, Lizcova L, Izakova S, Dostalova-Merkerova M, Siskova M, Jonasova A, Neuwirtova R, Cerna O, Cermak J and Michalova K: Recurrent chromosomal breakpoints in patients with myelodysplastic syndromes and complex karyotype versus fragile sites. Leuk Res. 2012 Jun;36(6):e125-7. **IF11 2.923**

*Hájková H, Marková J, Haškovec C, **Sárová I,** Fuchs O, Kostečka A, Cetkovský P, Michalová K, Schwarz J:* Decreased DNA methylation in acute myeloid leukemia patients with DNMT3A mutations and prognostic implications of DNA methylation. Leuk Res. 2012 Sep;36(9):1128-33. **IF11 2.923**

Abstracts with IF

*Brezinova J, Vcelikova S, Berkova A, Zemanova Z, Izakova S, **Sarova I**, Cechova H, Tajtlova J, Grosova L, Lizcova L, Malinova E, Zemanova M, Cmunt E, Karban J, Schwarz J, Michalova K:* Prognostic signifikance of telomere length, molecular cytogenetic findings and immunophenotypic features in patiens with B-chronic lymphocytic leukemia. 14th Congress of the European Hematology Association (Berlín, 4.-7.6.2009)
Abstrakt: Haematologica/The Hematology Journal Haematologica, 2009;94(s2):22.
IF09 6.416

Brezinova J, Berkova A, Zemanova Z, Izakova S, ***Sarova I****, Vcelikova S, Cechova H, Lizcova L, Cmunt E, Schwarz J, Michalova K:* Molecular cytogenetics, telomere length and other molecular and immunophenotypic features in patiens with B-chronic lymphocytic leukemia. The European Cytogenetics Association (Stockholm, 4.-7.7.2009)
Abstrakt: Chromosome Research, 2009;17(1):127. **IF09 3.230**

Zemanova Z, Michalova K, Brezinova J, Lizcova L, Izakova S, Bystricka D, ***Sarova I,*** *Berkova A, Siskova M, Neuwirtova R, Cerna O, Cermak J:* Frequency and Prognostic Impact of Complex Chromosomal Aberrations in Patients with Primary Myelodysplastic Syndromes and del(5q). The American Society of Hematology (ASH) (New Orleans, 2009).
Abstrakt: Blood 2009;114: 1623. **IF09 10.555**

Brezinova J, Vcelikova S, Berkova A, Zemanova Z, Izakova S, ***Sarova I****, Cechova H, Grosova L, Lizcova L, Malinova E, Cmunt E, Trneny M, Karban J, Schwarz J, Michalova K*: Telomere length evaluation in patiens with B-chronic lymphocytic leukemia – correaltion with other molecular, cytogenetic and immunophenotypic features.
The Europian Human Genetics (Gothenburg, 12.-15.6.2010)
Abstrakt: European Journale of Human Genetics, 2010;18(1):193-194. **IF10 4.380**

Brezinova J, Zemanova Z, Bystricka D, Izakova S, ***Sarova I****, Lizcova L, Merkerova-Dostalova M, Sponerova D, Jonasova J, Cermak J and Michalova K:* Combination of molecular cytogenetic techniques in mapping of the atypical 5q deletion in myeloid malignancies.
8th European Cytogenetics Conference (Porto, 2.-5.7.2011)
Abstrakt: Chromosome Research 2011;19(1):S133. **IF08 3.405**

Zemanova Z, Michalova K, Bystricka D, Brezinova J, Lizcova L, Izakova S, ***Sarova I,*** *Merkerová-Dostálová M, Siskova M, Zmolikova J., Cermak J:* True monosomy of chromosome 5 is presumably not an isolated cytogenetic entity in myelodysplastic syndromes (MDS).
8th European Cytogenetics Conference (Porto, 2.-5.7.2011)
Abstrakt: Chromosome Research 2011;19(1):S133. **IF11 3.087**

Brezinova J, Zemanova Z, Bystricka D, Izakova S, ***Sarova I****, Lizcova L, Malinova E, Sajdova J, Cermak J, Jonasova A, Michalova K:* Atypical deleted segments in three patiens with myeloid malignancies and deletion 5q.
16th Congress of the European Hematology Association (Londýn, 4.-7.6.2009)
Abstrakt: 16th Congress of the European Hematology Association, London, United Kingdom, June 9–12, 2011 Haematologica 2011 96(s2): 559. **IF11 6,532**

Zemanova Z, Michalova K, Bystricka D, Brezinova J, Lizcova L, Izakova S, ***Sarova I****, Merkerová-Dostálová M, Siskova M, Zmolikova J., Cermak J:* True monosomy of chromosome 5 is presumably not an isolated cytogenetic entity in myelodysplastic syndromes (MDS).
16th Congress of the European Hematology Association (Londýn, 4.-7.6.2009)

Abstrakt: 16th Congress of the European Hematology Association, London, United Kingdom, June 9–12, 2011 Haematologica 2011 96(s2): 600. **IF11 6,532**

Abstracts without IF

Zemanova Z, Bystricka D, Brezinova J, Lizcova L, Izakova S, ***Sarova I****, Dostalova-Merkerova M, Siskova M, Jonasova A, Neuwirtova R, Cerna O, Zmolikova J, Cermak J and Michalova K:* Genomic imbalances in 73 patients with MDS and complex karyotypes.
The 11th International Symposium on Myelodysplastic Syndromes (18.5.-21.5.2011, Edinburgh).
Abstrakt:
http://www.abstractserver.com/mds2011/planner/index.php?go=abstract&action=abstract_iplanner&absno=435&MDS2011=9vg1q8c2752nip7ektuv2unar2&MDS2011=9vg1q8c2752nip7ektuv2unar2.

Michalova K, Brezinova J, ***Sarova I****, Novakova M a Zemanova Z:* Presence of two different clones with deletion 5q in bone marrow cells of MDS patient.
6th Work Group meeting and 8th Management Committee meeting (Bělehrad, 23.-24.4.2012).

Březinová J, ***Šárová I,*** *Ransdorfová Š, Zemanová Z, Nováková M, Bystřická D, Sajdová J,Jonášová A, Šponerová D, Hrabánek J, Soukupová Maaloufová J, Čermák J, Michalová K:* Atypická místa zlomů v buňkách kostní dřeně nemocných s myeloidními malignitami.
XVI. Česko-slovenský hematologický a transfuziologický sjezd (Brno, 5.-8.9.2012)
Abstrakt: Sborník abstrakt 2012:94.

Schwarz J, Marková J, Berková A, Březinová, ***Šárová I*** *a Michalová K:* Transformace do sekundární akutní myeloidní leukemie u 2 pacientů s Ph myeloproliferativním onemocněním, spojená se ztrátou jedné alely genu TP53 a s mutací druhé alely. Leukemogenicita hydroxyurey?
XVI. Česko-slovenský hematologický a transfuziologický sjezd (Brno, 5.-8.9.2012)
Abstrakt: Sborník abstrakt 2012:137.