univerzitetni klinični center ljubljana
University Medical Centre Ljubljana

Detection of nucleophosmin (NPM1) gene mutations with different methods in patients with AML

Marija J. Mandelc Mazaj, Eva Surina, Uroš Mlakar, Tadej Pajič
University Medical Centre Ljubljana, Hematology Department, Zaloška 7, 1000 Ljubljana, Slovenia

INTRODUCTION

Mutations in exon 12 of the *nucleophosmin* (*NPM1*) gene are the most common genetic lessions found in acute myeloid leukemia (AML). They occur in about 50-60% of adult AML with normal karyotype. *NPM1* gene is located on chromosome 5q35 and encodes nuclear multifunctional proteins (Figure 1)[1].

Patients positive for mutations in *NPM1* gene and negative for internal tandem duplication mutations in FMS-like tyrosine kinase 3 (*FLT3*) gene have favourable prognosis in this group[1-5].

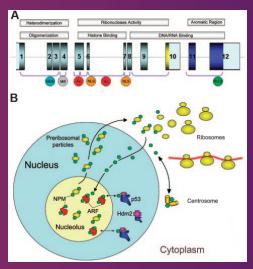

Figure 1. The NPM1 gene encodes for a protein involved in multiple functions[1].

THE AIM OF OUR STUDY

The aim of this study was to compare the results of the *NPM1* gene mutations detection by PCR-gel detection and sequencing of the amplified PCR products. Furthermore, we want to introduce the real-time quantitative polymerase chain reaction (RQ-PCR) assay for quantitative assessment of the most frequently observed mutations (type A and B).

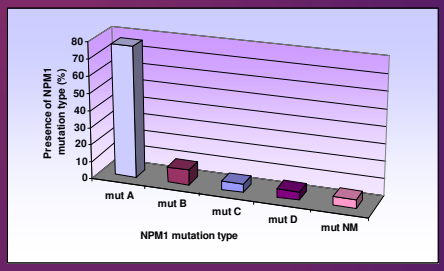

Figure 5. Detection of NPM1 mutation type with ABI PRISM 310 Genetic Analyser (Applied Biosystems).

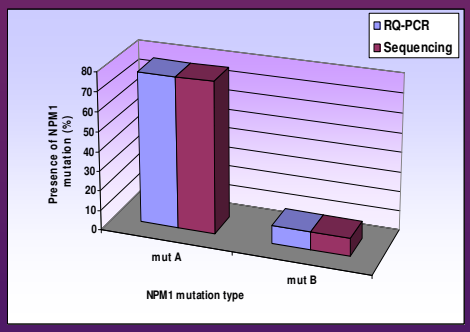

Figure 6. Detection of NPM1 mutation type A and B with RQ-PCR and sequence detection.

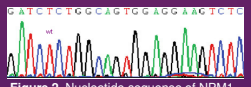

Figure 2. Nucleotide sequence of NPM1 wild type.

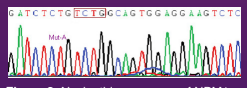

Figure 3. Nucleotide sequence of NPM1 mutation type A.

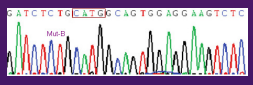

Figure 4. Nucleotide sequence of NPM1 mutation type B.

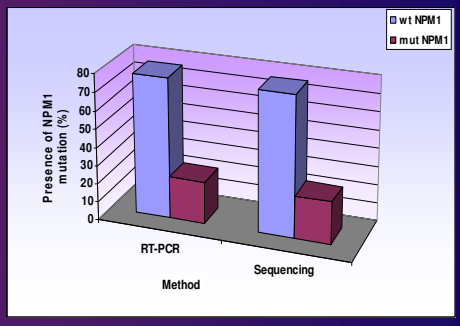

Figure 7. The concordance between PCR gel and sequence detection.

MATERIALS AND METHODS

Bone marrow samples from 95 patients with AML were analyzed. The diagnosis of AML was established Followed World Health Organization classification.

- Bone marrow aspirates were used for the isolation of mononuclear cells (MC) by ficoll density centrifugation.
- RNA was isolated from MC by High Pure RNA Reagent Kit (Roche).
- cDNA was performed by SuperScript II reverse transcriptase (Invitrogen).
- Amplification of the exon 12 of the *NPM1* gene was performed with the forward and reverse primer as described on Gene AMP® PCR System 9700 (Applied BioSystems)[4].
- The PCR products were visualized after agarose gel (4%) electrophoresis by ethidium bromide staining.
- All PCR-amplified samples were purified and sequenced on ABI PRISM 310 Genetic Analyser (Applied Biosystems).
- RQ-PCR reaction assay for mutation type A and B were performed with Muta*Quant*TM Standards Kit (Cancer Profiler, Ipsogen)1 on ABI PRISM 7000 SDS.

RESULTS

The concordance between PCR-gel and sequence detection was 100% (Figure 7). *NPM1* mutations were identified in 22 (23%) of the 95 AML patients. The most common detected mutation type was insertion of the TCTG tetranucleotide (type A, 77%), followed by insertion of the CATG (type B, 9%), CGTG (type C, 9%), CCTG (type D, 5%) and CCAG (type NM, 5%) (Figure 5). These are in agreement with recently published studies [1-5]. Results for mutation type A and B obtained with RQ-PCR assay were in agreement with results obtained with of PCR-gel and sequence detection. At RQ-PCR reaction assay we also performed inter and intra assay for reproducibility. High correlation coefficients (>0.99 in all experiments) allowed accurate assessment of NPM1 mutation A copies in unknown samples. Plasmid standard curves showed a mean slope of -3.37 and intercept of 40,01± 0,49 Ct. According to this assay, variation coefficient value was below 30%, which is in agreement with literature data.

	REPLICATES	PLASMID COPIES					SLOPE	R^2
		10	100	1000	100000	10000000		
Ct (exp.1)	1	37.28	32.86	29.76	23.61	19.99	-3.23	0.9951
	2	35.55	33.08	29.84	23.66	20.07		
Ct (exp.2)	1	36.93	33.35	29.68	23.15	19.53	-3.49	0.9992
	2	37.35	33.70	30.04	23.01	19.69		
Ct (exp.3)	1	37.01	33.35	29.82	23.44	19.81	-3.38	0.9995
	2	35.65	33.35	30.06	23.35	19.94		
Average Ct		36.63	33.28	29.87	23.37	19.84	-3.37	0.9979

Table 1. Reproducibility: results of experiments repeated three times. We tested 5 plasmid dilutions in duplicate. The plasmid contains nucleotide sequence of the NPM mutation A.

CONCLUSION

Our study shows, that PCR-gel detection is suitable screening method for identification of *NPM1* mutations. A sequence detection assay is suitable for confirmation of presence mutations. RQ-PCR was found out as sensitive and reliable method. On the basis of variation coefficient was also found out that RQ-PCR method is suitable for monitoring minimal disease in AML patients.

REFERENCES

[1] Brunangelo F., et al. Blood, 2007; 109: 874-885.
[2] Gorello P, et al. Leukemia, 2006; 20: 1103-1108.
[3] Verhaak RGW, et al. Blood, 2005; 106: 3747-3754.
[4] Döhner K, et al. Blood, 2005; 106: 3740-3746.
[5] Schlenk RF, et al. NJEM, 2008; 358: 1909-191.