

MINI-REVIEW

Prognostic Involvement of Nucleophosmin Mutations in Acute Myeloid Leukemia

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Abstract

Nucleophosmin (NPM1) is a protein of highly conserved nature which works as a molecular chaperone and is mostly found in nucleoli. NPM also involved in the maturation of preribosomes and duplication of centrosomes. Furthermore, it is also active in control and regulation of the ARF-p53 tumor suppressor pathway. A high rate of incidence and prognostic involvement is reported by various authors in AML patients. In AML it behaves as a favorable prognostic marker. NPM mutations are more frequently associated with normal-karyotype AML and are usually absent in patients having abnormal or poor cytogenetic. NPM mutations are not frequent in other hematopoietic tumors. Two main types of mutations have been described to date. Both of these cause abnormal cytoplasmic localization of NPM1. Their high incidence rate in normal karyotype and their favorable nature make those mutations hot spot or front face mutations which should be checked before treatment starts.

Keywords: Nucleophosmin - prognostic marker - AML - normal karyotype - NPM mutations - exon 12

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Introduction

In World Health Organization 2008, AML with mutated NPM have assigned a separate class of myeloid nucleoplasm which have unique molecular, prognostic and pathological feature (Michael et al., 2010). Various authors attribute mutated NPM as favorable prognostic marker for Overall survival or event free survival in AML having AML_NK. This attribution makes NPM an important candidate to study for the better understanding of leukemogenesis.

Structure of NPM Gene and Protein

Human nucleophosmin (NPM1) gene, is located at chromosome 5q35 and comprises of 12 exons (Chang et al., 1990). It is a phosphoprotein mainly present in nucleolus granular region, but shuttles continuously between nucleus and cytoplasm (Fallani et al., 2005). This shuttling is important in biogenesis of ribosome as well as in the transportation of preribosomal particles while in cytoplasm it binds to the unduplicated centrosome and regulates cell division (Fallini et al., 2005). NPM stabilizes genome via regulation of DNA repair process (Lee et al., 2005).

NPM has three isoforms (Falini et al., 2007) namely B23.1 (consist of 294 amino acid) and B23.2, (259 amino acid) and b23.3 (consists of 259 amino acids). The B23.1 is mostly present in all tissues (Fallini et al., 2007). The 35 amino acids long C-terminal of B23.1 is missing in B23.2 form while the 257 amino acid long, N-terminal of the

other splice variants are identical. The N-terminus region, performs multiple functions such as self-oligomerization and chaperone activity against histones, proteins, and nucleic acids (Okuwaki et al., 2000; Falini et al., 2007).

The nonpolar N-terminus region and multimeric region of NPM is essential and directly involved in the correct assemblage of maturing ribosomes in the nucleolus (Hingorani et al., 2000). The middle portion of NPM contains 2 acidic stretches which helps in histone binding (Ouwaki et al., 2001) while the in between fragment of the acidic stretches pertain ribonuclease activity. The C-terminus domain, binds with nucleic acid also have ribonuclease activity and is followed by short aromatic stretch which is critical for NPM binding to nucleolus (Hingorani et al., 2000).

NPM inhibits DNA fragmentation activity and plays a crucial role in hematopoietic stem cell modulation, regulation of DNA integrity and tumor suppressors genes p53 and ARF. NPM is undoubtedly important for balanced cell growth but the beneficial effects of NPM decreases upon maturity. The overexpression of NPM enhances chances of survival and recovery of hematopoietic stem cells under stress conditions on one hand while on other hand (Li et al., 2006) it promotes abnormal cell growth in malignant cells.

Interaction with p53

NPM regulates p53 levels and activity. There is a close association between NPM, "nucleolar integrity" and p53 stability (Colombo et al., 2002). In stress condition

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Table 1. Prognostic Importance/Clinical Out Come of *NPM1* Mutations in AML

| S | Name of Author | No of NPM cases | ITD status | Type of NPM mutation/ Insertion at nucleotide | Prognosis comments |
|----|-------------------------|-----------------|--|--|--|
| 1 | Kaseem et al., 2011 | 11/24 | Not mentioned | Type A Ins2(1015-1019CAGC)(2) Del (1178 (A))(7) 1base del (7) | Not mentioned |
| 2 | Krstovski et al., 2009 | 1/37 | +for FLT3D835 mutation | Type Q 4 bp insertion CGGA | Patient positive for FLT3/D835 and <i>NPM1</i> mutation relapsed |
| 3 | Thieda et al., 2006 | 9/75 | | Type A (1) Type B(6) Other (2) | All patients with <i>NPM1</i> mutations are alive in remission (except one, who died before starting treatment from cerebral bleeding). A comparison of the age of the adult patients with type A and non-type A mutations indicated that patients with type A mutations had a trend for a higher median age (60 years), compared to cases with non-A mutations (median age 57 years; p/0.068). |
| 4 | Brown et al., 2007 | 23/295 | | Type A (10) Other (13) | 18/23 NPMc+ received complete remission compared with 280NPM wildtype. survival data for 295 are not available while NPMc+ have good event free survival. |
| 5 | Mullighan et al., 2007 | 6/93 | | Type A (3) Type B (2) Type D (1) | Survival analyses did not detect associations between <i>NPM1</i> mutations and outcome, although this may be due to limited statistical power. The 6-year event-free survival was 55.6726.2 and 37.677.2% for those with and without the mutation, respectively (P/40.186) |
| 6 | Cazzaniga et al., 2005 | 7/107 | | Type A (1) TypeB (1) Type D (2) Type E (1) New (2) | All mutated patients achieved complete remission. None of the patients relapsed, 6 underwent bone marrow transplantation (BMT), and 5 of 7 are alive at last follow-up. |
| 7 | Shimada et al., 2007 | 0/33 | | Not mentioned | Not mentioned |
| 8 | Luo et al., 2010 | 31/57 | 12ITD+/19ITD- | NPM 1 mutation. Types not mentioned | favorable |
| 9 | Becker et al., 2010 | 83/148 | 33ITD+/50ITD- | 81% (Type A) 5%Type B) 4%(Type D) 11%(others) TCTG at position c.860-863 | <i>NPM1</i> mut patients had significantly better CR rate than <i>NPM1</i> wt patients. <i>NPM1</i> -mut patients also had a significantly longer OS compared with <i>NPM1</i> wt patients |
| 10 | Braoudaki et al., 2010 | 2/25 | | Type A mutation (1). Other mutation (1) TG mutation at codon 290, TC mutation at codon 293 | Complete remission of both NPM mutant patient* OneNPM + also bear t(8;21) (q22;q22). |
| 11 | Haferlach et al., 2009 | 328/576 | 515/576 | Not mentioned | No difference in survival was observed among <i>NPM1</i> -mutated AML patients independently of whether they carried a NK or an AK, the <i>NPM1</i> -mutated/FLT3-ITD negative cases showing the better prognosis EFS was significantly shorter in the <i>NPM1</i> -mutated/FLT3-ITD_ subgroup versus <i>NPM1</i> -mutated/FLT3-ITD_FLT3-ITD negative cases, no statistically significant difference emerged in OS and EFS of <i>NPM1</i> -mutated |
| 12 | Chou et al., 2006 | 33/173 | | 4 type of mutation. All patients with NPM mutations are heterozygous and have 4 bp insertions between nucleotides 960 and 961. | Not described |
| 13 | Micol et al., 2009 | 137/480 | | Not mentioned | Favorable outcome of npm in absence of FLT/ITD depend on presence of normal karyotype. |
| 14 | Boonthimat et al., 2008 | 105/400 | 56.8% | Type A (81) Type B (5) Type D (7) Type J (2) Type DD-4(1) Novel (8) | NO major difference in the overall survival (OS) in the Thai patients with and without <i>NPM1</i> mutation (p=0.376). |
| 15 | Milos et al., 2012 | 20/73 | | Type A was found in 18 patients, and the remaining two patients had type D and type K mutations. | The lowest CR rate was detected in the <i>NPM1</i> +/FLT3+ double positive group (3/11; 27.3%), followed by the <i>NPM1</i> + / FLT3- (4/9; 44.4%) and <i>NPM1</i> -/FLT3+ (4/8; 50%) single positive groups, while the highest CR rate was found in the <i>NPM1</i> -/FLT3- double negative group (36/45; 80%). Kaplan-Meier analysis of DFS revealed significant differences only between the <i>NPM1</i> - / FLT3 - (16 months) and <i>NPM1</i> - / FLT3+ groups (8 months) |
| 16 | Boissiel et al., 2005 | 50/106 | | Not mentioned | Of the 106 patients, 92 (87%) achieved CR after induction therapy. there is no difference in CR rates between NPMm and NPMwt patients (86% versus 88%; p 0.99). |
| 17 | Lin et al., 2006 | 99 | | 5 type of NPM mutation | Favorable |
| 18 | Brown et al., 2007 | 23/295 | 52ITD+ 218ITD- | 43% Type A 9% Type B 9% Type D 4% Type F 4% Type J 8 novel cases reported | 78% of the patients with NPMc achieved a CR compared with 85% patients without NPM. Thus, <i>NPM1</i> mutation status did not significantly affect induction CR rate |
| 19 | Taussig et al., 2010 | 27 | 13ITD+ | 20/27 (TCTG) type) 4/27(CCCTG) 1/27(CATG)) 1/27(CCTG) 1/27(CCCTG) | Not mentioned |
| 20 | Gorello et al., 2006 | 15 | ITD not mentioned | 9/15(type A)) 2/15(TypeB) 2/15(TypeD) | Not mentioned |
| 21 | Kazem et al., 2011 | /2650 | Not mentioned | Type not mentioned | No relation between cNPM positivity and age, sex, cytoplasmic positivity for NPM was significantly correlated with increased survival and better outcome after cycles of chemotherapy. |
| 22 | Dohner et al 2005 | 145/300 | | Type A (76%) Type B (8%) Type D (7%) 12 Novel | The highest remission rate was achieved in the <i>NPM1</i> mutated /FLT3 ITD negative group (86%), followed by the <i>NPM1</i> -unmutated/FLT3 ITD-positive group (76%) and the group without mutations (68.5%); the lowest response rate (63%) was achieved in the <i>NPM1</i> mutated/FLT3 ITD-positive group (p 0.001). |
| 23 | Ammatuna et al., 2005 | 8/56 | | duplication of TCTG tetranucleotide at positions 956-959(typeA) nucleotides 965-969 (GGAGG) were substituted by the 9mer GCTTTAGTC. | Not mentioned |
| 24 | Michela et al 2007 | 11/28 | 16/28 have ITD/ TKD either at diagnosis or relapse*** | 9/28hasTCTG 2/11hasCCCTG | Not mentioned |
| 25 | Roti et al 2006 | 26/120 | NMntd | 9/26 typeA 5/26typeB 5/26typeD 7/26 others 4/7 new variants [ins964_965(GCTT); 965G_C; [ins963-964(AGGA)] [ins964_965(CTCT); [ins959_960(GCCA)]. | Not mentioned |

Table 1 (continue). Prognostic Importance/Clinical Out Come of *NPM1* Mutations in AML

| S | Name of Author | No of NPM cases | ITD status | Type of NPM mutation/ Insertion at nucleotide | Prognosis comments |
|----|-------------------------|-----------------|----------------------|---|---|
| 26 | Michael et al 2010 | 9/17 | | 6has typeA 1type 1typeD 2typeJ | Not mentioned |
| 27 | Fallini et al 2005 | 166/591 | 59/209 ITD+ | Not mentioned | 71 %had complete remission after induction therapy |
| 28 | Tan et al 2008 | 12/44 | 8/44 | 11/44TCTG(Type A) 1/44CTGC | Not mentioned |
| 29 | Tiziana et al., 2008 | 52 | Not mentioned | 21/52 type A (detected by allele specific) | Not mentioned |
| 30 | Todd et al., 2008 | 17/70 | Not mentioned | 12 type 2 typeB 2typeD lnew mutation864-865delGCinsCTGGCG | Not mentioned |
| 31 | Szankasi et al., 2008 | 9/33 | | 8/9 typeA 1/9Nm type | Not mentioned |
| 32 | Dalea et al., 2011 | 34/71 | 17/34ITD+ | | DFS and OS did not differ between mutated and unmutated NPM patients. A difference in outcome (OS) was observed between NPM+ Flt3- and NPM+Flt3+ 82.4% versus 76.5% without reaching a statistical significance |
| 33 | Verhaak et al., 2005 | 95/275 | | Type A (72) Type B (12) Type D (4) Type I (1) Type J (1) Type K (1) 12 Novel 4undetermined variants | The EFS, OS and probability of relapse at 60 months for the AMLpatients with or without <i>NPM1</i> mutations were similar |
| 34 | Suzuki et al., 2005 | 64/257 | | Type A (49) Type B (7) Type D (4) Novel (4) | The CR rate was significantly higher in the patients with <i>NPM1</i> mutations (42 of 49; 85.7%) than without them (97 of 141; 68.8%) (p 0.025). |
| 35 | Schnittger et al., 2005 | 212/401 | | Type A (166) Type B (13) Type D (21) Other (12) | In <i>NPM1</i> -mutated cases, CR rates were significantly higher (70.5% vs 54.7%, p 0.003); EFS was significantly longer (median, 428 vs 336 days; p 0.012). Median OS showed a trend toward better prognosis (1012 days vs 549 days; p 0.076) EFS was significantly longer (median, 428 vs 336 days; p 0.012) |
| 36 | Li et al., 2006 | 20/99 | | Type A (13) Type B (2) Type D (3) Other (2) | Not mentioned |
| 37 | Yangjeon, 2012 | 19/83 | | Type A (16) Type B (1) Novel (2) | Not mentioned |
| 38 | Elizebeth, 2012 | 158/284 | | Type A (117) Type B (16) Type D (9) Type G (3) Other (13) | Not mentioned |
| 39 | Ruan et al., 2008 | 36/220 | | Not mentioned | |
| 40 | Ahmad et al., 2009 | 39/200 | | Type A (69.2%) Type B (5.1%) Type D (15.3%) Type H (2.5%) Type Nm 2.5%) Npvel (2) | Not mentioned |
| 41 | Qin et al., 2008 | 11/35 | 4/35=for itd and npm | Not mentioned | Not mentioned |
| 42 | Roel et al 2005 | 69/252 | | Not mentioned | intermediate cytogenetic risk group without FLT3 ITD mutations and with <i>NPM1</i> mutations have a significantly better OS/EFS than those without <i>NPM1</i> mutations |

nucleolus behaves as a stress sensor where NPM plays an important role to arrest “p53 dependent cell cycle” (Kurki et al., 2004).

Interaction with ARF

NPM and ARF both mostly localize in the nucleolus. (Bertwistle et al., 2004). They interact in a mutually beneficial way. Hence, NPM prevent ARF from destruction while ARF control NPM polyubiquitination (Kuo et al 2004; Grisendi et al., 2005). Although this interactions is still in debate but under cellular stress condition, NPM and ARF are reorganized to the nucleoplasm (Gjerset et al., 2006).

Frequency and stability of *NPM1* mutations

In the un translated region of *NPM1*, at position 1146 a deletion of T nucleotide was observed in majority (60-70%) of AML patients and in healthy volunteers. *NPM1* mutations retain a wild-type allele and is usually in heterozygous state. Their frequency ranges 2.1% (Taiwan), 6.5% (European countries) which accounts about 9-26.9% in infantile AML-NK (Cazzaniga et al., 2005; Chou et al., 2006; Mullighan et al., 2007) while in adults it ranges upto 25-35%, which accounts 45.7-63.8% of adult AML-NK 80-83, 90, 93, 94 (Falini et al., 2005; Lin et al., 2006; Falini et al., 2007; Kassem et al., 2011).

NPM1 mutations are more stable than FLT3 mutations and contrary to FLT3 mutations, they are also present at relapse while NPM negative patients at presentation cannot

acquired them at relapse or during the malignancy which indicates that NPM mutations are not directly involved in advancement of disease. Lin et al. (2006) reported the mutually exclusive nature of NPM and CEBPA. In some of the cases, the loss of *NPM1* mutations was typically responsible for the transformation of normal karyotype into abnormal karyotype (Suzuki et al., 2005; Chou et al., 2006).

Discovery and various types of *NPM1* mutations in AML

The first attempt to detect NPM mutation was done by GIMEMA/AML12 EORTC trial. This trial screened 591 AML-NK patients and observed cytoplasmic NPM (NPMc). The sequencing of above mentioned cases confirmed mutations in exon 12. After this, no of studies confirmed that Frame shift mutations in exon 12 result in loss of a nucleolar localization signal and halt movement of mutant protein to the cytoplasm (Brown et al., 2007; Fallini et al., 2008 ; Kim et al., 2010; Matson et al, 2010; Kaseem et al., 2011). More than 40 different *NPM1* mutations in exon 12 have been identified in AML and all are highly constrained to exon 12, except two mutations which involves exon 9 and exon 11 (Albiero et al., 2006; Mariona et al., 2006). In majority, NPM mutations harbor type A mutations (75-80%) while type B and type D comprises (10%), and (5%) mutations respectively (Falini et al., 2007; Szankasi et al., 2008; Kaseem et al., 2011). These four mutations represent about 90-95% of all *NPM1*-positive cases (Hafeez et al., 2010) and 60% of

them have normal karyotype AML (Brown et al., 2007). Table 1 has shown recent information about various types of NPM mutation.

Role of NPM mutation in promoting leukemia

NPM mutations are rarely seen with chromosome abnormalities and are more common in normal karyotypes, this is the indication that they play preliminary role in the process of leukemogenesis (Colombo et al., 2005; 2006). However, it is still unclear that how the mutant protein propagates leukemia. Uptill now various studies reported that all NPM mutations result in abnormal dislocalization of the mutant protein into the cytoplasm which causes "leukemogenesis" (Bolli et al., 2007; Falini et al., 2009). Accelerated transport of nucleophosmin into cytoplasm probably triggers "multiple cellular pathways" by "loss of function"/or "gain of function". It also causes the interaction of NPM mutant protein to others proteins which are present in cytoplasm. Although no study still confirms that this interaction causes "leukemogenesis" but it was reported that mutant protein involved in "knock down the oncosuppressor ARF" (denBesten et al., 2005; Colombo et al., 2006) and activates c-MYC oncogene (Bonneti et al., 2008). Further more, dislocation of mutant protein causes lessen amount of wild-type *NPM1* in the nucleolus which result in dislocation into cytoplasm by the formation of heterodimers with *NPM1* mutant..and loss of heterozygosity. NPM heterozygous cells are more susceptible to "oncogenic transformation" (Falini et al., 2009). One study in NPM knockout mouse reported that, NPM inactivation creates genomic instability which, promotes cancer susceptibility *in vitro* and *in vivo* (Falini et al., 2011).

Conclusion

NPM mutations are presently the most prevailing mutations in AML-NK. In absence of other genetic abnormalities they have proven their crucial prognostic importance in intermediate risk group. These mutations provide favorable response and better overall survival in absence of Internal Tandem Duplication (ITD) in AML patients. Hence ITD-/NPM+ genotype have shown over all better response in AML_NK.

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