MINI-REVIEW

Prognostic Involvement of Nucleophosmin Mutations in Acute Myeloid Leaukemia

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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 karyoptype 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

Asian Pac J Cancer Prev, **14 (10)**, 5615-5620

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 *NPM1* mutation relapsed
3	Thieda et al., 2006	9/75		Type A (1)) Type B(6)) Other (2)	All patients with *NPM1* 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; p¼0.068).
4	Brown et al., 2007	23/295		Type A (10) Other (13)	18/23 NPMc+ recived complete remission compred 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 *NPM1* 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 (P¼0.186)
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	*NPM1*mut patients had significantly better CR rate than *NPM1*wt patients. *NPM-1*mut patients also had a significantly longer OS compared with *NPM1*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 *NPM1*-mutated AML patients independently of whether they carried a NK or an AK, the *NPM1*-mutated/ FLT3-ITD negative cases showing the better prognosis EFS was significantly shorter in the *NPM1*-mutated/FLT3-ITD_ subgroup versus *NPM1*-mutated/ FLT3-ITD_FLT3-ITD negative cases, no statistically significant difference emerged in OS and EFS of *NPM1*-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	Fvorable 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 *NPM1* 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 *NPM1* +/ FLT3+ double positive group (3/11; 27.3%), followed by the *NPM1*+ / FLT3– (4/9; 44.4%) and *NPM1*–/ FLT3+ (4/8; 50%) single positive groups, while the highest CR rate was found in the *NPM1* –/ FLT3– double negative group (36/45; 80%). Kaplan-Meier analysis of DFS revealed significant differences only between the *NPM1* – / FLT3 – (16 months) and *NPM1*– / 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, *NPM1* mutation status did not significantly affect induction CR rate
19	Taussig et al., 2010	27	13ITD+	20/27 (TCTG) type) 4/27(CCTG) 1/27(CATG)) 1/27(CTTG) 1/27(CCTG)	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 *NPM1* mutated /FLT3 ITD negative group (86%), followed by the *NPM1*-unmutated/FLT3 ITD–positive group (76%) and the group without mutations (68.5%); the lowest response rate (63%) was achieved in the *NPM1*mtated/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 2/11hasCCTG	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 1new 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 *NPM1* 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 *NPM1* 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 *NPM1*-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	Elizebath, 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 *NPM1* mutations have a significantly better OS/EFS than those without *NPM1* 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 kayotypes, 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 abnormalies they have proven their crucial prognostic importance in intermediate risk group. These mutations provide favorable response and better overall survival in absence of Internal Tendom Duplication (ITD) in AML patients. Hence ITD-/NPM+ genotype have shown over all better response in AML_NK.

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