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Brief Communication

Can probability of genetic mutation be an indicator of clinical relevance?

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Abstract

NPM1 gene mutation evaluated on a population basis is a valuable and realistic tool to reflect the pathophysiological relevance of cancer. In a comparison of the *NPM1* cDNA of human bladder cancer with its consensus sequence, we have found that a higher *NPM1* sequence identity in a population is consistent with poor tumor differentiation, advanced tumor stage, and likelihood of recurrence. These data imply that "probability" of *NPM1* mutation is an indicator of status of malignancy.

Keywords: Nucleophosmin; Population genetics; Probability of mutation; Genetic variations; Gene identity

Genetic variations in the human genome range from a single nucleotide to megabases in size. While copy number variations (CNVs) are widely seen in human cancers [1–5], single-nucleotide polymorphisms (SNPs) may also play a role in individual disease susceptibility [6]. Unlike the association of CNVs with phenotype change, accumulation of nucleotide changes in a gene in a population has not been well delineated.

Nucleophosmin (NPM; also known as B23, NO38, or numatrin) is a multifunctional nucleolar phosphoprotein. NPM has crucial roles in proliferation and homeostasis, such as ribosome biogenesis, centrosome duplication, cell cycle progression, and cell differentiation [7,8].

NPM is important in apoptosis and modulation of the ARF–p53 tumor suppressor pathway [9]. With the intriguing biological function as an oncogene or a tumor suppressor, the gene dosage, expression level, and genetic mutation of *NPM1* have been examined extensively [10].

In hematological malignancy, *NPM1* translocations and the resulting fusion proteins are often found. Patients with anaplastic large cell lymphoma (ALCL) with *NPM1*/*ALK* t(2;5)(p23;q35) translocation show excellent response to induction therapy and are prone to better prognosis [11]. *NPM1* exon 12 and cytoplasmic NPM mutant ($NPMc^+$) in acute myelogenous leukemia

Table 1
Patient characteristics

	No.	%
Patients	43	
Patient age (years)		
Median	69	
Range	35–90	
Sex		
Male	33	76.7
Female	10	23.3
T stage		
pT1	29	67.4
pT2	5	11.6
pT3	8	18.6
pT4	1	2.3
Histologic grade		
1	15	34.8
2	18	41.9
3	10	23.3
Primary tumor	26	60.5
Recurrent tumor	17	39.5

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(AML) represent subgroups with better treatment response and prognosis [12,13]. The genetics underlying the pathogenesis of solid tumor, however, is poorly defined.

Over 90% of bladder cancers are transitional cell carcinomas that have recurrence rates as high as 50–70%. About 20% of recurrences progress to invasive cancer. Metastatic bladder cancer is incurable and associated with poor prognosis. Dyrskjot et al. have reported the molecular signature of superficial bladder cancer with a propensity for invasion [14]. The majority of these genes are involved in the regulation of apoptosis, cell differentiation, and cell cycle progression. We previously have shown that NPM mRNA overexpression is associated with advanced tumor stages and grades [15]. This prompts us to investigate the possible *NPM1* genetic correlation with its clinical relevance. Since an event of cancerous transformation is the final result of multiple deregulations, a single genetic change may not be enough to reflect to phenotype. It thus becomes conceivable and important for us to evaluate genetic changes in *NPM1* on a population basis.

Results

With the approval of the Institutional Review Board for the Protection of Human Subjects, 43 patients with bladder transitional cell carcinoma diagnosed from 2002 to 2004 at Chang Gung Memorial Hospital were recruited. Patients' ages ranged from 35 to 90 years (median 69). Thirty-three (76.7%) patients were male and 10 (23.3%) were female. Twenty-six (60.5%) patients had primary and 17 (39.5%) had recurrent bladder cancers. The pathological grading was 15 (34.8%) grade 1, 18 (41.9%) grade 2, and 8 (23.3%) grade 3 tumor. Twenty-nine (67.4%) patients were pT1, 5 (11.6%) were pT2, 8 (18.6%) were pT3, and 1 (2.3%) was pT4 stage (Table 1).

Tumors with similar pathological or clinical setting were segregated for alignment to the consensus RefSeq cDNA sequence of *NPM1*. With the algorithm of ClustalW applied in the alignment analysis of vector NTI, the gene identity was quantified. Fig. 1 shows the complexity of *NPM1* in each population. The shaggy appearance at the end of each bar denotes the variation in the population alignment (Fig. 1A).

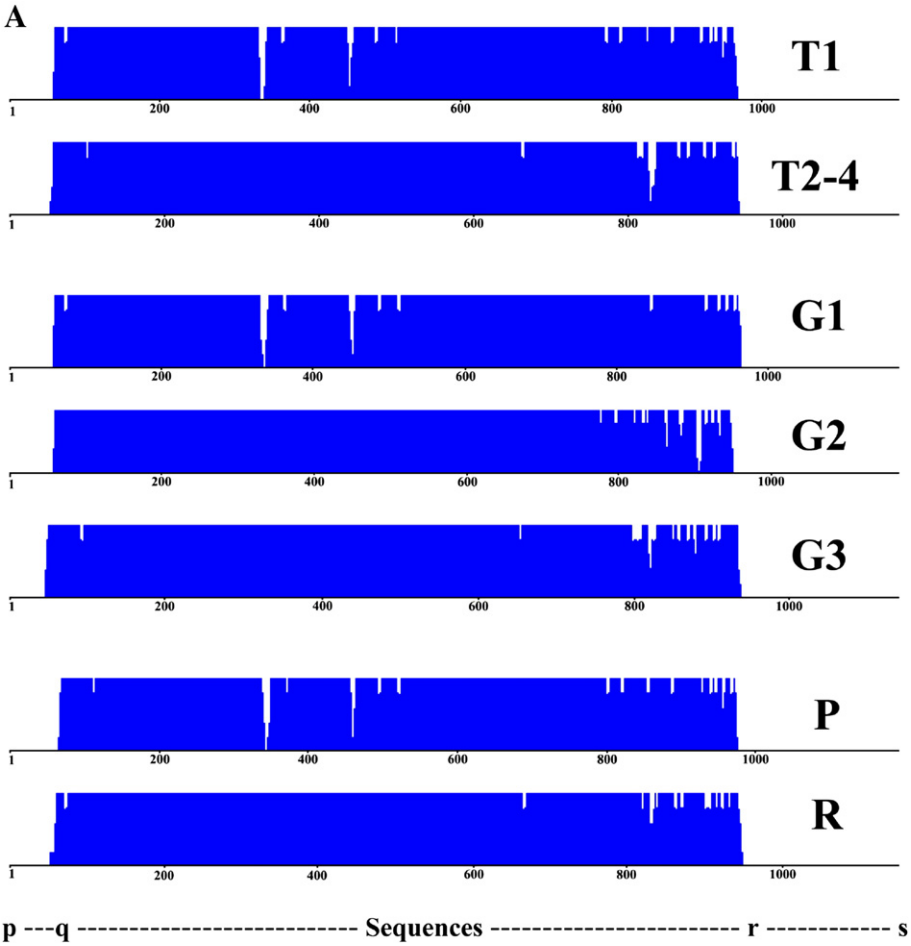


Fig. 1. *NPM1* population alignment. (A) *NPM1* complexity in different clinicopathological settings. Bladder tumors of pathological superficial stage (T1), invasive stage (T2–4), histological grade (G1, G2, G3), and primary (P) or recurrent (R) are assorted for alignment. Sample number in each group is shown in Table 1. p and s, start and end of cloned sequences; q and r, start and end of alignment to consensus *NPM1* cDNA. (B) *NPM1* alignment at the C_terminus. The top shows tumors of pathological stage T1 and the bottom those of advanced stages (T2–T4). At the top of each group is the consensus *NPM1* sequence. Yellow color denotes identical alignment in all specimens. α, arbitrary sample names; β, clinical/pathological information; γ, group alignment.

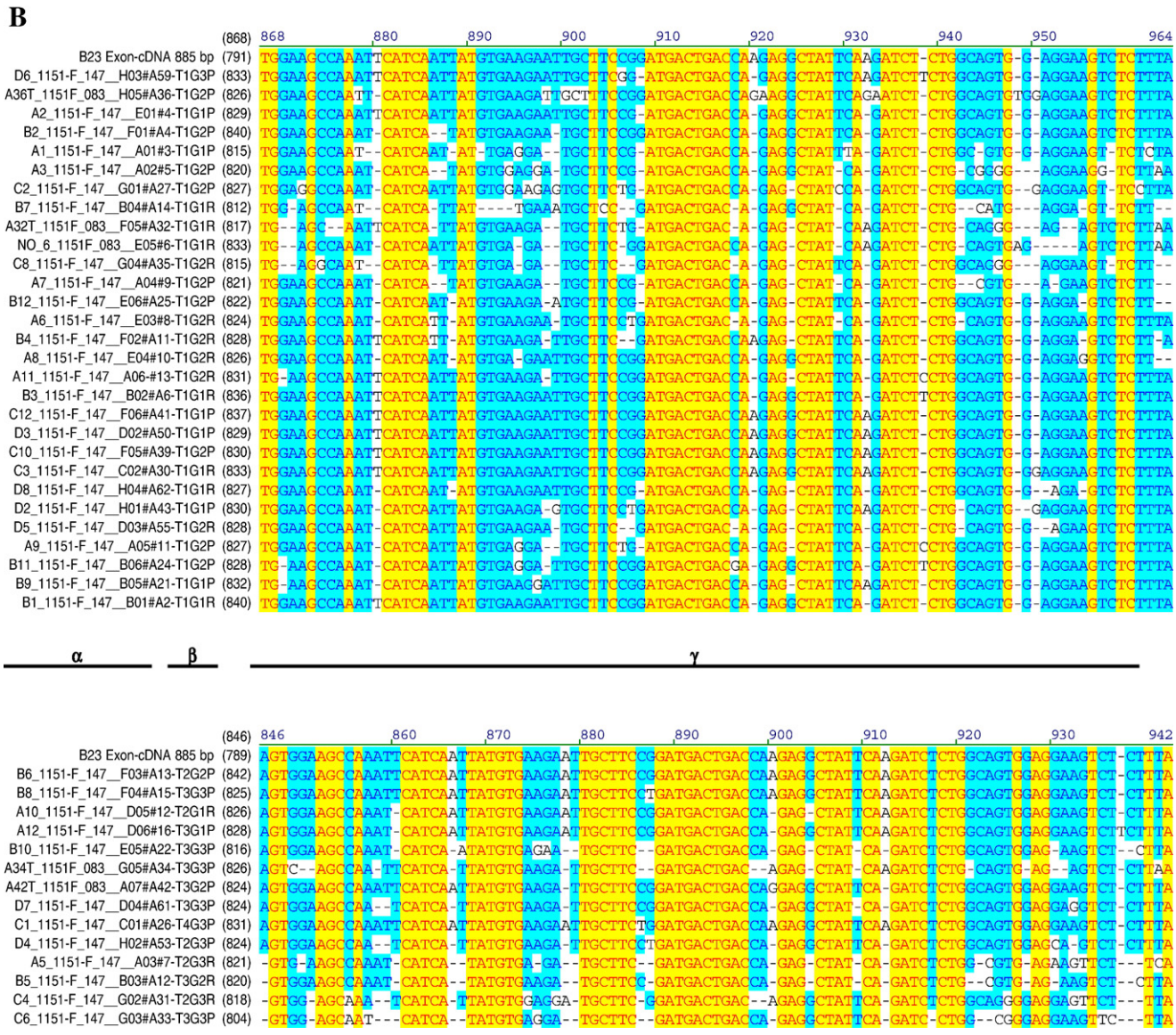
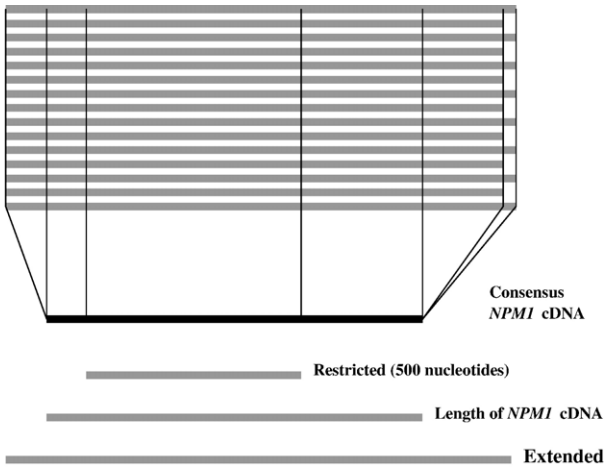


Fig. 1 (*continued*).

This might also reflect the frequent mutation propensity of *NPM1* at the C-terminus (Fig. 1B). The identity of *NPM1* with the consensus in the pT1 population was 66.4%, whereas it was 88.7% in the population with invasive tumor (pT2–4). The population gene identity in the group of pathological grade 1 was 70.5% and that in grades 2 and 3 was 87 and 89.5%, respectively. The overall gene identity in primary tumor in this cohort was 66.8%, while it was 88.3% in the population with recurrent tumors (Fig. 2). Furthermore, in shorter sequence comparison (restricted to 500 nucleotides), the sequence could be highly identical to the consensus sequence (up to 99.8%). The comparison can also be made to encompass the full cloning sequences (extended comparison). The trend of higher *NPM1* sequence identity appearing in tumors of high malignancy was well preserved (Fig. 2). Bladder cancer with advanced tumor stage, higher tumor grade, and higher status of recurrence shows higher sequence identity to the consensus *NPM1* cDNA. In this context, *NPM1* in bladder cancer acts more like a proto-oncogene. The integrity of *NPM1* shows increased probability of completion of tumor behavior. Accumulation of genetic variation results in defective malignancy potential and this can be seen in either tumor staging or grading pathologically and status of recurrence clinically. The concept of evaluating tumor behavior on a population basis in relevance to the sequence identity of a crucial cancer gene such as *NPM1* will give us a novel view in tumor genetics.

Discussion

Cancer is, in essence, a genetic disease [16]. Recent advances in biotechnology such as microarrays enable researchers to perform high-throughput comparisons of multiple genes between different biological settings or conditions. Thousands of genes with minor differences may be responsible for each



	NPM1	Restricted	Extended
T stage			
pT1	66.4	66.8	50.9
pT2-4	88.7	99.8	68.5
Histologic grade			
1	70.5	67	54.4
2	87	99.6	66.3
3	89.5	99.8	69.7
Primary tumor	66.8	67.6	50.8
Recurrent tumor	88.3	99.6	68.5

Fig. 2. *NPM1* identity at different sequence lengths. Using *NPM1* cDNA as the alignment profile, higher *NPM1* sequence identity in a population is consistent with poor tumor differentiation, advanced tumor stage, and likelihood of recurrence. This trend is seen in comparison to *NPM1* cDNA and well preserved at either restricted length (500 nucleotides) or extended comparison.

clinical variation. The concept may be straightforward in such divergent comparisons. Herein, we propose the convergent comparison of a gene in a population by its identity to the consensus sequence in different pathological and clinical settings. We suspect that a gene that might work with our hypothesis would be a pivotal gene involved in major cellular activity. *NPM1* is a transcription target of the proto-oncogene *Myc* [17]. NPM regulates the stability and transcriptional activity of p53 [9]. p53 has been linked to about half of human cancers and its mutation has prognostic implications in bladder cancers [18]. As a member of the nucleoplasmin family, NPM acts as a molecular chaperone that shuttles rapidly between nucleus and cytoplasm. With the diversity of its binding to many partners, it is involved in much more complex biological activity than expected.

One may expect that a tumor is the result of a genetic defect that causes cell cycle deregulation and escape from the surveillance of a tumor suppressor gene such as *p53*. One may also be interested in finding possible clues to identify subgroups in all cancer patients that will be more susceptible to treatment outcome and better prognosis. Falini et al. reported that mutation in the carboxy-terminus of *NPM1* causes encoding of abnormal proteins that are crucial for the nuclear export signal motif and nucleolar localization [19]. These mutants cause cytoplasmic localization of NPM. $NPMc^{+}$ represents a subgroup of patients who have normal karyotype and better prognosis. In fact, *NPM* exists in normal cells. Under proliferating or tumorigenesis conditions, *NPM* will over-express in response to the proliferative process or oncogenic stress. Mutation in a pivotal gene and functional protein in cancer cells could be associated with better treatment response and prognosis.

It is well known that the strongest predictors in a clinical scenario are tumor stage and grade. In this study, using a finite alignment algorithm, the *NPM1* identity in a population correlates well with bladder tumor stage, grade, and recurrence status. Our data imply that the probability of *NPM1* mutation is an indicator of clinical relevance in bladder cancer. Most importantly, *NPM1* genetic changes evaluated on a population basis prove to be valuable and realistic in reflecting the pathological and clinical settings of cancer. *NPM1* is illustrated to be a crucial gene in cancer genetics.

Materials and methods

Total RNA was extracted using the Trizol method (Invitrogen). Fresh mRNAs were reverse transcribed to cDNA using M-MLV (Invitrogen). Both forward and reverse primers spanning the *NPM1* gene were analyzed to confirm the fidelity of sequence. The forward primer was 5′-GCAGCGTTCTTT-TATCTCCG-3′, about 37 bp upstream of the transcription start site. The reverse primer was 5′-TAAAACCAAGCAAAGGGTGG-3′, about 180 bp downstream of the transcription stop site. Primers were designed to encompass the full length of *NPM1* and for the fidelity of full-length sequencing of the gene. The consensus sequence used was from NCBI (NM_002520) with the *NPM1* sequence of 885 nucleotides. Sequences analysis for alignment, using *NPM1* as the profile, was performed using Vector NTI Suite 8 (InforMax).

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