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SHORT COMMUNICATION

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Isolation and characterization of a novel human *NM23-H1B* gene, a different transcript of *NM23-H1*

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Abstract The *NM23* gene is a conspicuous metastasis-suppressor gene. Eight human genes of the NM23/nucleoside diphosphate kinase family have been discovered. From our large cDNA cloning and sequencing project, we cloned a different transcript (*NM23-H1B*) of human *NM23-H1* from 18-week-old human fetal brain. The 987-bp cDNA encodes a protein of 177 amino acid residues. Compared with *NM23H1*, the cDNA contained an additional NH_2-terminal region (25 amino acid residues). It was mapped to chromosome 17q21.3 using bioinformatics analysis, which shows that the second exon does not exist in *NM23-H1*. The expression pattern of *NM23-H1B* showed that it was ubiquitously expressed in normal tissues (15 tissues except colon) at different levels. Our data also indicated that the expression of the transcript in tumors related to tumor differentiation: in poorly differentiated breast carcinoma GI-101, pancreatic adenocarcinoma GI-103, and undifferentiated ovarian carcinoma GI-102, there was no expression. In poorly differentiated lung carcinoma LX-1, lung carcinoma GI-117, the expression level was very low. The transcript band in well-differentiated colon adenocarcinoma CX-1 was significantly higher than that in poorly differentiated colon adenocarcinoma GI-112. A high transcription level was also found in grade IV prostatic adenocarcinoma PC3.

Key words NM23-H1B · NM23-H1 · Transcript · MTC panel and PCR · 17q21.3

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Introduction

The *NM23* gene is a conspicuous metastasis-suppressor gene. Expression of the gene correlated inversely with metastatic potential (Steeg et al. 1988). Bevilacqua et al. (1989) found that *NM23* RNA levels were differentially expressed in human breast tumors and that low *NM23* RNA levels were associated with histopathologic indications of high metastatic potential. NM23 is a heterodimeric protein that acts as a nucleoside diphosphate (NDP) kinase (Gilles et al. 1991) that catalyzes phosphoryl transfer from a nucleoside triphosphate to an NDP. It is ubiquitously found in organisms from bacteria to human. NDP kinases are involved in the synthesis of nucleoside triphosphates, and the NM23 protein may act in the regulation of signal transduction by complexing with G proteins, causing activation/inactivation of developmental pathways (Stahl et al. 1991). Eight human genes of the NM23/NDP kinase family have been discovered from analysis of their sequences (Lacombe et al. 2000).

The two most widely studied genes, *NM-23H1* and *NM23-H2*, encode the A and B polypeptide chains of the enzyme. Each chain consists of 152 amino acid residues. The cDNA of *NM23-H2* predicts a protein of molecular weight 17 kDa, with 88% identity to *NM23-H1*. Northern blot hybridization indicated that the expression of *NM23-H2* was reduced to a lesser metastatic potential in tumor cells than that of *NM23-H1*. Both proteins are independently active NDP kinases and readily form intra- and intermolecular disulfide bonds (Stahl et al. 1991). Both *NM23-H1* and *NM23-H2* were assigned to 17q21.3 by somatic cell hybrid analysis and fluorescence in situ hybridization (Backer et al. 1993) and were 4 kb apart on the chromosome (De la Rosa et al. 1995).

Materials and methods

A high-quality cDNA library was constructed with a modified pBluescrip II SK (+) vector by using 18-week-old human fetal brain mRNA (Clontech, Palo Alto, CA, USA).

A 0.5-kb DNA fragment containing Sfi (5′-GGCCATTA TGGCC-3′) and SfiB (5′-GGCCGCCTCGGCC-3′) recognition sites was cloned into *Eco*RI and *Not*I sites of pBluescript II SK (+) (Stratagene, La Jolla, CA, USA); the modified vector was then digested by *Sfi*I and the large fragment was excised and purified for library construction. A cDNA library was constructed by following the SMART polymerase chain reaction (PCR) cDNA library construction kit protocol (Clontech). The cDNA inserts were sequenced on an ABI PRISM 377 DNA sequencer (Perkin-Elmer, San Francisco, CA, USA) using the BigDye Terminator Cycle Sequencing Kit and BigDye Primer Cycle Sequencing Kit (Perkin-Elmer) with −21M13 primer. An M13Rev primer and synthetic internal walking primers were designed according to the obtained cDNA sequence fragments. Each part of the inset was sequenced at least three times bidirectionally. Subsequent editing and assembly of all the sequences from one clone was performed using Acembly (Sanger Centre Cambs, UK). From our large-scale sequencing analysis of human cDNA libraries, we cloned a full-length cDNA encoding a homologue of the mouse *NM23* gene and human *NM23-H1*. The cDNA consisted of 987 bp and contained an open reading frame (ORF) of 534 bp (from 238 bp to 771 bp) encoding a protein of 177 amino acids. The cDNA was considered to be full length because there was an upstream in-frame stop codon (TAA) and a Kozak sequence. The nucleotide sequence has been submitted to the Genbank/EMBL Database with accession number AF487339.

Results

Bioinformatics analysis using BLASTn (http://www.ncbi.nlm.nih.gov/BLAST) showed that from 89 bp to 305 bp of AF487339 do not exist in *NM23-H1* (BC000293, BC018994, NM-000269, X17620, BC008015, AL360166, AL360191, X73066). Interestingly, BLASTp revealed that the cDNA contained an additional NH_2-terminal region (25 amino acids), whereas the rest was the same as *NM23-H1*. There was a casein kinase II phosphorylation site motif (SSCD; 19–22 amino acids) in the additional NH_2-terminal region (http://www.expasy.ch/prosite). Various levels of homology with *NM23-H2*, *NM23-H3*, *NM23-H4*, *NM23* (mouse), and *NM23* (rat) were also detected (Fig. 1).

To determine the chromosomal localization of AF487339, we used the international human genome

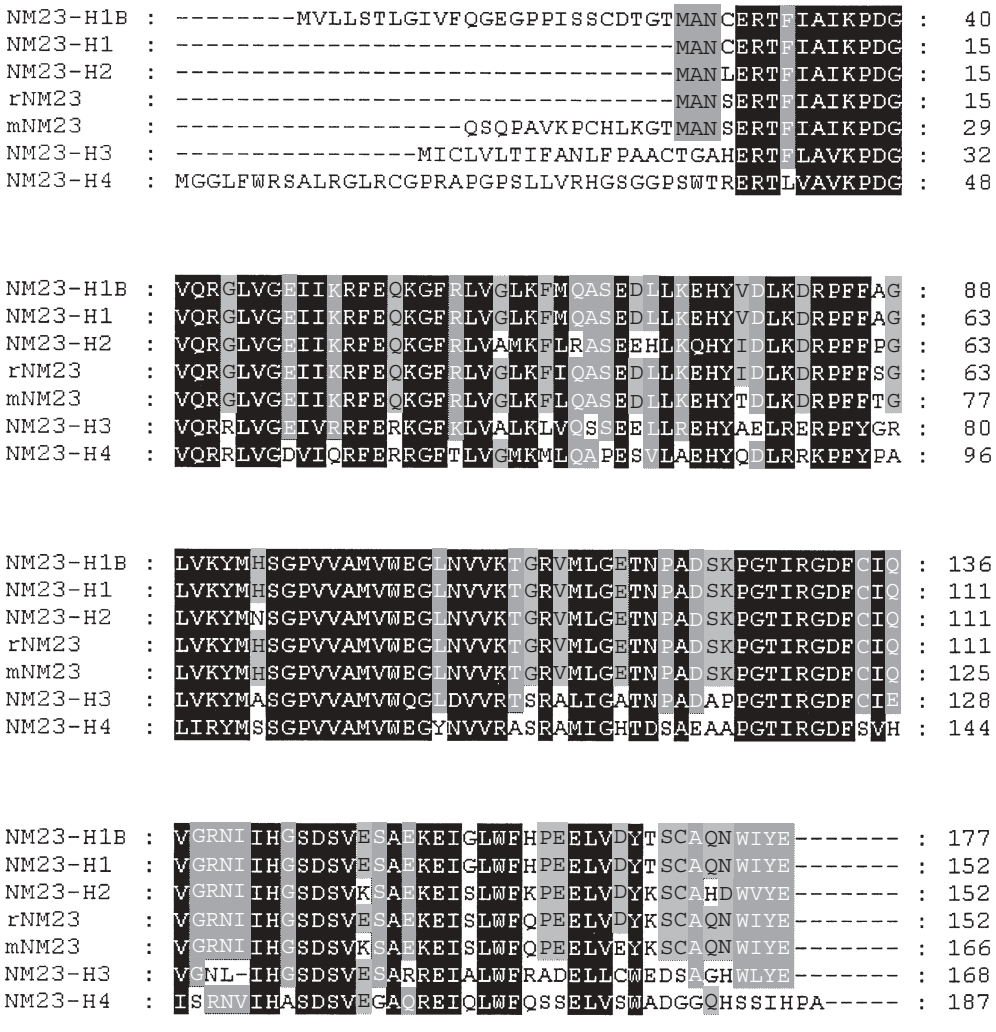

Fig. 1. Alignment of *NM23-H1B* with *NM23-H1*, *NM23-H2*, *NM23-H3*, *NM23-H4*, *NM23* (mouse), and *NM23* (rat). *Numbers on the right* refer to the last amino acid in each corresponding line. Identity is indicated by a *black box*, and similarity is indicated by a *gray box*

Table 1. Nucleotide sequence of exon–intron junctions of the *NM23-H1B* gene

3′ Splice acceptor	Exon	Size (bp)	5′ Splice donor	Intron	Size (bp)
CDNAend GTGCAAGTGCTG	1	87	GCAGCTGGAAGG**gt**aagaggtgtt	1	562
attgactgct**ag**GCCCTGTGGCTA	2[a]	220	CTGTGATACAGG**gt**aggtcatgag	2	1206
gcctatcccc**ag**AACCATGGCCAA	3	130	AAATTCATGCAA**gt**aagtggactt	3	4199
cctgttgaat**ag**GCTTCCGAAGAT	4	102	GTAGTTGCCATG**gt**gagtgtgcct	4	1078
ctgtccttgg**ag**GTCTGGGAGGGG	5	113	ACAAGTTGGCAG**gt**gagattttgg	5	455
ttctccaccc**ag**GAACATTATACA	6	334	TTGGTTACTTCA		

The intron sequence is shown in lowercase letters and the exon sequence in uppercase letters
[a] Exon 2 does not exist in *NM23-H1* cDNA

database at NCBI (http//:www.ncbi.nlm.nih.gov/blast). The gene was mapped to contig NT-010783.8, spanning 8486 bp. The contig was located at 17q21.3 with the same site as *NM23H-1*. Comparing our cDNA with the genome sequence suggested that the gene had six exons and five introns. All sequences at the exon–intron junctions were consistent with the AG–GT rule (Table 1). The ORF was in exon 2 to exon 6. It revealed that the second exon of AF487339 does not exist in *NM23-H1*. Other genes located nearby were glial fibrillary acidic protein (17q21), homeo box B13 (17q21.2), DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 5 (RNA helicase, 68 kDa) (17q21), and nucleotide-binding protein (17q12–q21). We considered that AF487339 was a different transcript of *NM23-H1* (which we termed *NM23-H1A*) and we assigned AF487339 to *NM23-H1B*.

To further verify that *NM23-H1B* was a different transcript than *NM23-H1A* and to confirm the difference of expression between *NM23-H1B* and *NM23-H1A*, tissue distribution was determined by multiple tissue cDNA (MTC) panel (Clontech) and human tumor panel (Clontech) as PCR templates according to the manufacturer's protocol. On the basis of bioinformatics analysis, we designed specific primers, including the ORF of the transcript, so that the results of PCR should be *NM23-H1B* sequences and not *NM23-H1A* sequences. The sequences for *NM23-H1B* specific primer pairs were 5′-CAGGAGTAAATCAGCCTG GTGTGCAG-3′ (F, from 121 bp to 146 bp, which do not reside on *NM23-H1A* cDNA) and 5′-GAGAACTCACAG CTCCAAGAGCTTCC-3′ (R, from 883 bp to 908 bp). The sequences for *NM23-H1A* specific primer pairs were 5′-GTTCAAACCTAAGCAGCTGGAAG-3′(F) and 5′-CTCCTGTCATTCATAGATCCAGTTCTGA-3′(R). The prospective transcript band of *NM23-H1B* was 788 bp and that of *NM23-H1A* was 493 bp. Thirty-six cycles of amplification (30 s at 94°C, 1 min at 60°C, and 1 min at 72°C) were performed using ELONGGASE DNA polymerase (Gibco Brl, Gaithersburg, MD, USA). The PCR product of the transcript was then resolved on 1.5% Metaphor agarose gel (FMC, Philadelphia, PA, USA). In total, 16 normal human tissues and 8 human tumor tissues were tested and the specific bands were accorded a prospective length (Fig. 2a). The data showed that *NM23-H1B* was ubiquitously expressed in normal tissues (15 tissues except colon) at different levels. In the human tumor panel, we found the specific bands of *NM23-H1B* in colon adenocarcinoma CX-1, colon adenocarcinoma GI-112, lung carcinoma LX-1, lung carcinoma GI-117, and prostatic adenocarcinoma PC3 (Fig. 2b). The specific bands of *NM23-H1A* were found in heart, brain, placenta, lung, liver, skeletal muscle, pancreas, spleen, and thymus, whereas no specific bands of *NM23-H1A* could be detected in kidney, prostate, testis, ovary, small intestine, and peripheral blood leukocytes (Fig. 2a). In the human tumor panel, we found specific bands of *NM23-H1A* in poorly differentiated lung carcinoma GI-117, poorly differentiated colon adenocarcinoma GI-112, and undifferentiated ovarian carcinoma GI-102 (Fig. 2b).

Discussion

NM23-NDP Kinases are distributed ubiquitously, although their expression may vary in a differentiation- and tissue-specific manner. Early studies showed that NM23 was implicated in cell proliferation, differentiation, and cancer. NM23-H1 is a potential negative regulator of growth factor genes, whereas NM23-H2 is a positive regulator of the c-myc gene, itself crucial to cell growth and differentiation (De la Rosa et al. 1995; Postel et al. 1993). Current work shows that the human NDP kinase genes are differentially expressed in tissues and that their products are targeted to different subcellular locations. Data suggest that NM23/NDP kinases possess different, but specific, functions within the cell, depending on their localization (Lacombe et al. 2000). Tumor suppressor genes have a pivotal role in normal cells, regulating cell cycle processes negatively. Furthermore, the inhibition of cell proliferation is a crucial step in the achievement of cell differentiation. Increasing evidence suggests that the *NM23* genes, initially documented as suppressors of the invasive phenotype in some cancer types, are involved in the control of normal development and differentiation (Lombardi et al. 2000).

We report here a different transcript (*NM23-H1B*) of human *NM23-H1A* from our large cDNA cloning and sequencing project. The gene is 987 bp long and encodes a protein of 177 amino acid residues, which is the same as the NM23H1A protein except for an additional NH_2-terminal region (25 amino acids). Bioinformatics analysis indicates that the *NM23-H1B* gene is mapped to chromosome 17q21.3. It has six exons and five introns, but the second exon is missing in *NM23-H1A*. For tissue distribution, our

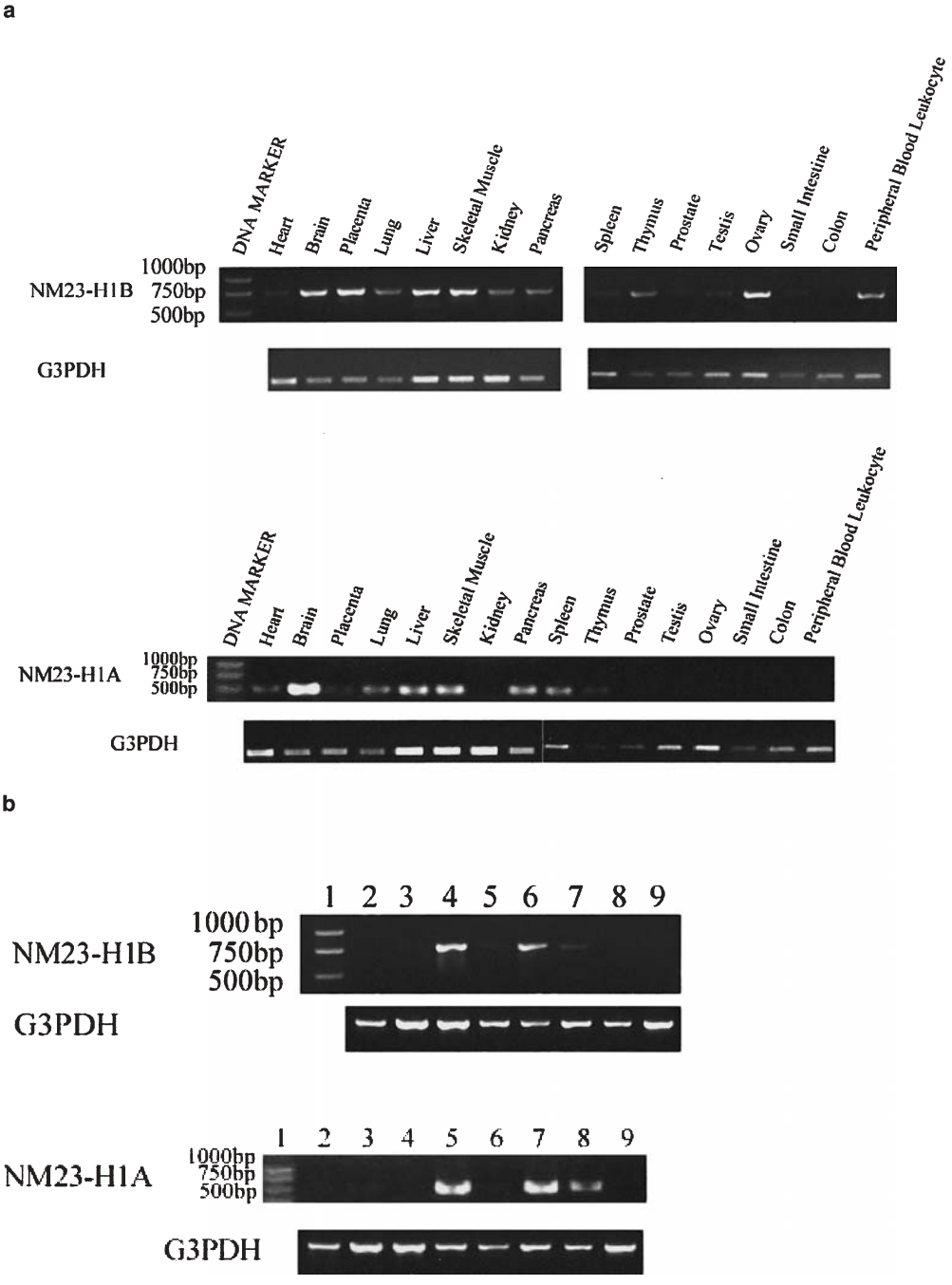

Fig. 2. a Normal tissue distribution of NM23-H1B and NM23-H1A. *G3PDH*, Glucose-3-phosphate dehydrogenase. **b** Tumor tissue distribution of NM23-H1B and NM23-H1A. *Lane 1*, DNA marker; *lane 2*, breast carcinoma GI-101, poorly differentiated mammary carcinoma isolated from recurrent ductal carcinoma; *lane 3*, lung carcinoma LX-1, poorly differentiated carcinoma, surgical explant from metastasis from a 48-year-old man; *lane 4*, colon adenocarcinoma CX-1, well-differentiated adenocarcinoma consistent with gastrointestinal origin; *lane 5*, lung carcinoma GI-117, poorly differentiated carcinoma, established from a tumor of a 62-year-old woman; *lane 6*, prostatic adenocarcinoma PC3, grade IV adenocarcinoma from a 65-year-old Caucasian; *lane 7*, colon adenocarcinoma GI-112, moderately to poorly differentiated adenocarcinoma, established from a 54-year-old woman; *lane 8*, ovarian carcinoma GI-102, undifferentiated carcinoma isolated from a primary ovarian carcinoma; *lane 9*, pancreatic adenocarcinoma GI-103, poorly differentiated carcinoma, propagated from ascitic fluid from a pancreatic adenocarcinoma

data reveal a transcript in brain, placenta, liver, skeletal muscle, ovary, and peripheral blood leukocytes. Expression is lower in heart, lung, kidney, pancreas, spleen, thymus, prostate, testis, and small intestine. No specific band can be detected in colon. Our data also indicate that the expression of *NM23-H1B* is related to tumor differentiation: in poorly differentiated breast carcinoma GI-101, pancreatic adenocarcinoma GI-103, and undifferentiated ovarian carcinoma GI-102, there is no expression. In poorly differentiated lung carcinoma LX-1, lung carcinoma GI-117, the expression level is low. The transcript band in well-differentiated colon adenocarcinoma CX-1 is significantly higher than that in poorly differentiated colon adenocarcinoma GI-112, but it is also expressed highly in grade IV prostatic adenocarcinoma PC3. We also detected the expression of *NM23-H1A*. Our data showed that there is a difference between *NM23-H1B* and *NM23-H1A* in human normal panel and in human tumor panel. Further studies will focus on the protein function of *NM23-H1B*.

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