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Identification of a Second Human nm23 Gene, nm23-H2

John A. Stahl,1 Alvaro Leone, Ariella M. Rosengard, Laura Porter,2 C. Richter King,2 and Patricia S. Steeg3

Laboratory of Pathology, National Cancer Institute, NIH, Bethesda, Maryland 20892 [J. A. S., A. L., A. M. R., P. S. S.], and Molecular Oncology, Incorporated, Gaithersburg, Maryland 20878 [L. P., C. R. K.]

Abstract

Reduced RNA and/or protein levels corresponding to the murine nm23-1 and human nm23-H1 complementary DNA clones have been correlated with high tumour metastatic potential in several rodent model systems and human breast carcinomas. We report the identification of a second human nm23 gene, designated nm23-H2. The pNM23-H2S complementary DNA clone predicted a Mr, 17,000 protein 88% identical to nm23-H1. nm23-H2 also shared a significant homology with nucleoside diphosphate kinases and a Drosophila developmental gene. Southern blots containing Bgl II-restricted genomic DNA, which exhibited an allelic restriction fragment length polymorphism for nm23-H1, contained nonallelic bands upon rehybridization to the nm23-H2 probe. Thus, nm23-H1 and nm23-H2 are distinct genes. Northern blot hybridization of nm23-H1- and nm23-H2-specific probes to breast tumours and cell lines indicated that nm23-H1 expression was reduced in high metastatic potential tumour cells to a greater extent than nm23-H2. The data indicate the existence of a family of independently regulated nm23 genes.

Introduction

Tumor metastasis is the major cause of death for cancer patients (1). Even without evidence of metastatic disease at the time of primary tumor diagnosis, patients face a 50% chance that clinically occult micrometastases exist and will become manifest (2). Accordingly, considerable investigation has focused on the genetic events that regulate tumor metastasis, in an effort to identify better diagnostic and therapeutic strategies. In this regard, the nm23 gene was identified by differential colony hybridization between related low and high metastatic murine K-1735 melanoma lines (3). Quantitative reductions in nm23 RNA and/or protein levels have been observed in the more highly metastatic tumour cells in four rodent metastasis model systems: murine K-1735 melanomas (3); N-nitrosomethylurea-induced rat mammary tumours (3); mouse mammary tumor virus induced tumours (4); and ras ± adenovirus 2 Eia cotransfected rat embryoblasts (5). In a limited series of primary human infiltrating ductal carcinomas, tumours from patients with metastases to the lymph nodes at surgery contained lower nm23 RNA levels than did nonmetastatic primary tumours (6). Two nm23 cDNA clones have been characterized, murine pNM23-1 and human pNM23-H1 (7), which both encode Mr, 17,000 nuclear and cytoplasmic proteins. The function(s) of nm23 are unconfirmed, but significant homologies
GCG CG
nm23-H2S
CGG CCA CGA GGC GGA ATC CCT TCT GCT CCA CAG CCG CCG CCT CCA CCA

Ang Arg Arg Arg Glu Cys Asp Ser Ser Leu Pro Ala Glu Arg Arg Arg Pro Asp Pro Asp

GCG CG
nm23-H1
GGG GGG GGG CTC TGG TCC CGG CCT CGG CCG CTC TC TCT GCG CAG CTG CTG

Glu Glu Glu Glu Cys Cys Cys Pro Pro Ser Cys Pro Pro Pro Pro Pro Pro

IDENTIFICATION OF nm23-H2

The translation region of murine nm23-H2, resulting in the identification of the nearly full length human nm23-H1 (7) and a distinct pNM23-H2 cDNA clone. The nucleotide sequence of pNM23-H2 (Fig. 1) bases 202–670 suggested that it encoded the COOH terminus of a distinct, nm23-like protein.

A 419-base pair Fnu4HI-HindIII restriction fragment of pm23-H2 was used to screen a human lung fibroblast Lambda Zap II phage library, resulting in the identification of the pNM23-H2 cDNA clone. The sequence of the pNM23-H2 insert, determined byideoxy sequencing of both strands, is compared in the previously identified nm23-H1 (7). Methionine with an optimal translation initiation sequence was boxed; for pm23-H2, this methionine has been demonstrated to be the initiation point for translation (7). Predicted amino acids which are identical in pm23-H1 and pm23-H2 are dotted.

Previously,3 Blots were hybridized with either the nm23-H1 or nm23-H2 probes at 40°C, in 5x SSC, 50 mm Tris, pH 7.5, 5x Denhardt’s, 0.2% (w/v) SDS-50 µg/ml sheared, denatured salmon sperm DNA for 16 h. Blots were washed sequentially in 2, 1, 0.5, and 0.25x SSC; 0.2%(w/v) SDS; and 1 mM EDTA at 55°C and then again in the last buffer 0.2% (w/v) SDS-50 µg/ml sheared, denatured salmon sperm DNA for 16 h. Blots were washed sequentially in 2, 1, 0.5, and 0.25x SSC; 0.2%(w/v) SDS; and 1 mM EDTA at 55°C and then again in the last buffer.

Results

Identification of the pNM23-H2 and pNM23-H2S cDNA Clones. A human fibroblast cDNA library was screened with

Fig. 2. Amino acid sequence similarities between nm23-H2, other nm23 proteins, NDP kinases and Drosophila a/wd. The predicted amino acid sequences of pNM23-H2S, human pNM23-H1 (7), murine pNM23-I (3), Drosophila abdominal wing discs (awd) developmental gene (7), rat NDP kinase (8), Dictyostelium NDP kinase (9), and Myxococcus NDP kinase (10) are listed. The percentages of nm23-H2 amino acid identity to each protein, calculated based on the number of amino acid residues which overlap, are listed below. Leucines in a configuration consistent with a leucine zipper (14) are boxed.

Fig. 3. nm23-H1 and nm23-H2 are distinct genes. Human genomic DNA from six individuals was digested with BglII, and a Southern blot was prepared. The blot was hybridized with the BamHI restriction fragment of pNM23-H1 (A). An allelic pattern of hybridizing nm23-H1 bands was obtained, including homozygotes for the 7.6-kilobase band (Lanes 1 and 2), homozygotes for the 2.3-kilobase band (Lanes 3 and 4), and heterozygotes (Lanes 5 and 6). The probe was removed from the Southern blot, and it was rehybridized to the EcoRI restriction fragment of pNM23-H2S (B). Bands distinct from the alleles of nm23-H1 were observed at 17.5, 13.8, 4.2, 2.8, and 2.4 kilobases. Sizes of DNA bands were calculated from the electrophoretic mobility of DNA λ HindIII markers.

\[ \text{nm23-H2} \]  
\[ \text{nm23-H1} \]  
\[ \text{awd} \]  
\[ \text{NHF, rat} \]  
\[ \text{NHF, alme mold} \]  
\[ \text{NHF, bacteria} \]

\[ \text{nm23-H2S} \]  
\[ \text{nm23-H1} \]  
\[ \text{awd} \]  
\[ \text{NHF, rat} \]  
\[ \text{NHF, alme mold} \]  
\[ \text{NHF, bacteria} \]

\[ \text{awd} \]

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Discussion

The predicted amino acid sequences of nm23-H1 and nm23-H2 have prompted at least two hypotheses concerning their biochemical functions. First, nm23-H2 exhibited a 97% amino acid sequence identity to the rat NDP kinase. Barring the possibility that the four amino acids changed between nm23-H2 and rat NDP kinase abrogated its activity, it is anticipated that nm23-H2 is a NDP kinase. We are currently in the process of expressing and purifying the nm23-H1 and nm23-H2 proteins from bacteria to confirm their NDP kinase activities. The NDP kinases are thought to provide intracellular pools of nucleoside triphosphates (excluding ATP), regulate polymerization of microtubules in the mitotic spindle and cytoskeleton, and supply GTP to G-proteins in signal transduction (16). It is not known if nm23-H1 and nm23-H2, if proven to be NDP kinases, participate in different functions.

The second proposed functional domain for nm23-H2 (and nm23-H1) is a leucine zipper, a motif proposed to mediate protein:protein binding and found on nuclear oncogenes and transcription factors (14). The nm23 proteins, awd, and rat NDP kinases exhibit 2–3 leucines in a characteristic arrangement, short of the 4 leucines in the classic definition of a leucine zipper (14). However, leucine zippers of 2–3 residues have been reported in other proteins (15). It is noteworthy that the leucine repeat is an evolutionary addition, in that the lower eukaryotic and prokaryotic NDP kinases do not exhibit this motif. This observation also suggests that protein dimerization via a leucine zipper is not necessary for NDP kinase activity. A region containing basic amino acids was identified to the NH2 terminus of each leucine repeat, although the placement of basic amino acids within this region did not fit a proposed DNA binding site model (15). The data suggest a possible function of nm23-H1 and nm23-H2 as transcription factors. Consistent with this hypothesis is the localization of nm23 protein in both the cytoplasm and nucleus (7). Proof of this hypothesis awaits evidence of homo- or heterodimerization of nm23 and identification of a DNA sequence that is bound and regulated.

On the basis of the high degree of identity between nm23-H1 and nm23-H2 throughout their translated regions, we conclude that previously published data using full length probes may have measured the composite RNA level for both genes. In this report we have quantitated specific expression of nm23-H1 and nm23-H2 in a limited series of breast carcinomas and cell lines, using probes to their 3’ untranslated regions and stringent washing conditions. On these Northern blots, nm23-H2 RNA levels were not reduced in the more highly metastatic specimens to the extent that nm23-H1 RNA levels were. The data indicate that the two genes are not identically regulated. The data should not be generalized to indicate that nm23-H2 expression is not inversely correlated with tumor metastatic potential. In a series of v-Ki-ras-transfected, immortalized human bronchial epithelial cell lines (17), the expression of nm23-H2 was decreased with increasing malignant behavior to a greater extent than that of nm23-H1. The data indicate the independent regulation of the two nm23 genes and suggest that factors such as cell type and oncogene expression may be relevant to further dissection of this complicated regulatory network.

References


IDENTIFICATION OF \textit{nm23-H2}


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