Expression of *Nm23* in Gliomas and its Effect on Migration and Invasion *In Vitro*

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Abstract. *Background: Despite the well-documented importance of Nm23 in the control of metastasis, there is currently a paucity of data regarding the role of this gene family in the control of glioma invasion. Materials and Methods: Nm23-H1 expression in gliomas was assessed via immunohistochemistry, Western blot, RT-PCR and Northern blot analyses. The migration and invasion ability were also investigated in primary glioma culture cells, human glioma cell lines and nm23-H1 transfectant, using an in vitro brain slice invasion model and a simple scratch technique. Results: Although no significant correlations were detected between nm23-H1 expression and pathological grade, the endogenous nm23-H1 expression in gliomas was found to be inversely correlated with their migratory abilities. Additionally, the nm23-H1 transfectant resulted in a reduction of approximately 45% of the migratory ability and suppressed the invasiveness of the parental cell line. Conclusion: Our overall findings suggest that nm23-H1 may play an important role in the suppression of glioma invasion and migration.*

The primary cause of local recurrence and therapeutic failure in the treatment of malignant gliomas is the invasion of tumor cells into the surrounding normal brain tissues. Consequently, anti-invasive strategies can be viewed as a novel and important adjunctive approach to glioma therapy. Invasion is defined as the ability of cells to penetrate the extracellular environment and achieve the active translocation of neoplastic cells through the host extracellular matrix (ECM) barriers (1-3). The invasion process is characterized by a complex series of steps, involving the alteration of the tumor cells' interaction with specific ECM ligands, the proteolysis of the matrix by hydrolytic enzymes and the subsequent migration of cells through the degraded structures (1-3). The infiltrative nature of high-grade gliomas is responsible for much of the morbidity and mortality which is associated with these tumors. Surgical debulking of the tumor is often only a temporary measure, as microscopically-infiltrated foci of a tumor will eventually lead to recurrence, often in areas that are surgically inaccessible.

Metastasis suppressor genes are characterized by their inhibition of tumor cell line metastatic capability upon *in vivo* transfection, without inhibiting primary tumor size. *Nm23* is a recently investigated putative metastasis suppressor gene, which has demonstrated interesting properties with regard to cell invasion and metastasis. The *nm23* gene family, which is currently recognized as having eight members, is known to exhibit metastasis suppressor activity. Transfection of *nm23* cDNAs into metastatically competent breast carcinoma, melanoma, colon carcinoma and oral squamous cell carcinoma cell lines resulted in a 40～98% reduction of metastatic potential *in vivo* (4-7). The *nm23* gene encodes for nucleoside diphosphate (NDP) kinase, which exhibits, in some tumors, a metastatic suppressor effect (5, 7-11). Down-regulation of *nm23* mRNA levels has been observed in a variety of cells with a high potential for metastasis (8, 11-14).

Despite the well-documented importance of *nm23* in the control of metastasis, there is a paucity of data regarding the role of this gene family in the control of brain tumor invasion. Gliomas are the most commonly observed primary brain tumors. In their malignant form, they are characterized by rapid cellular proliferation and diffuse brain invasion, both of which contribute to an overall poor prognosis. The purpose of the present study was to

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investigate the expression of *nm23* in gliomas, as well as its effect on migration and invasion *in vitro*.

Materials and Methods

Astrocytoma cell lines, primary cell cultures and culture conditions. The human malignant astrocytoma cell line U87 MG (obtained from the American Type Culture Collection, Rockville, MD, USA) and primary culture cells were grown in monolayer culture. The cell line was routinely maintained in alpha minimal essential media (α-MEM) (Gibco BRL, Gaithersburg, MD, USA) supplemented with 10% fetal calf serum (FCS) and 100 U/ml penicillin, 100 mg/ml streptomycin and 26 mg/ml amphotericin B (Gibco). Low- and high-grade human glioma specimens were obtained during craniotomy. Primary cultures from the specimens were taken by mechanical dissociation and maintained under the same culture conditions as above. Specimens of normal human brain were also obtained during the course of routine craniotomies performed on trauma or epilepsy patients.

Protein preparation and Western blot analysis. The glioma tissue was homogenized with a Polytron homogenizer at 3,000 rpm in a solution containing 250 mM sucrose, 1 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride and 20 mM potassium phosphate buffer, at pH 7.6. Large tissue debris and nuclear fragments were removed by two low speed spins in succession (3,000 xg, 5 min; 10,000 xg, 10 min). The protein concentration was determined by the method of Bradford with bovine serum albumin as a standard (15). Western blotting was done according to the method of Mattson and Higgins (16) with a slight modification. Protein samples (30 μg) were electrophoretically size-separated with a discontinuous system consisting of a 15% polyacrylamide resolving gel and 5% polyacrylamide stacking gel, and transferred to a nitrocellulose membrane at 20 V and 100 mA overnight. The membranes were washed, blocked, incubated with a 1:1,000 dilution of polyclonal rabbit anti-nm23-H1 (Santa Cruz Biotechnologies, CA, USA), and then incubated with a horseradish peroxidase-labelled goat anti-rabbit IgG (1:4,000). The bound secondary antibody was detected by enhanced chemiluminescence (Amersham, Buckinghamshire, England). The protein levels were determined by analyzing the signals of autoradiograms using the transmitter scanning videodensitometer.

Reverse transcription polymerase chain reaction (RT-PCR). Total RNA was isolated from the glioma according to the protocols of the Ultraspec™ RNA isolation system (Biotecx), then the polymerase chain reaction (PCR) was applied. For the reverse transcription (RT) step, along with the reaction mixture recommended by the enzyme manufacturer, 1 μg total RNA was incubated with 200 U of reverse transcriptase (Gibco BRL, Grand Island, NY, USA), 10 mM dNTP mix, 0.1 M DTT, 25 mM $MgCl_2$, oligo (dT) (0.5g μg/ml), and reaction buffer [200 mM Tris HCl (pH 8.4), 500 mM KCl] in a final volume of 20 μl. After a final denaturation step at 70˚C for 15 min, 2 μl cDNA was subjected to PCR consisting of denaturation at 94˚C for 5 min. PCR cycles were performed in a thermal cycler (M.J. Research, Watertown, MA, USA) with the following profile: denaturation for 1 min at 94˚C, annealing 1 min at 60˚C for nm23-H1 primers and 1-min extension step at 72˚C; denaturation 45 sec at 94˚C, annealing 45 sec at 56˚C for β-actin primers and 1 min 30 sec extension step at 72˚C. The last cycle was ended with 7 min of elongation at 72˚C. β-actin primers were as previously reported (17). The *nm23-H1* cDNA was amplified using primers (sense primer, 5'-GCGTACCT TCATTGCGATCAAAC-3': and antisense primer: 5'-ATCCAG TTCTGAGCACAGCTCGTG-3'), allowing for the amplification of 435 bp fragments. The *β-actin* cDNA was amplified using primers (sense primer: 5'-GACTACCTCATGAAGATCCTGACC-3': antisense primer: 5' -TGATCTTCAT GGTGCTAGGAGCC-3') allowing for the amplification of 423 bp fragments. The PCR contained 20 pmole of each primer, 250 μm dNTP mix, 1.5 mM $MgCl_2$, 40 mM KCl reaction buffer [50 mM Tris-HCl (pH 8.3)], and 1U of Taq polymerase (Bioneer, Korea) in a final volume of 20 μl. The PCR products were size-fractionated by 1.5% agarose gel electrophoresis, and visualized under UV light with ethidium bromide staining. The *nm23-H1* and *β-actin* cDNA were quantified by IMAGERTM & 1D MAIN (Bioneer). The *nm23-H1* cDNAs were normalized by comparison with cDNA.

RNA isolation and Northern blot analyses. Total RNA was isolated from the glioma according to the protocols of the Ultraspec™ RNA isolation system (Biotecx), quantitated by spectrophotometer at 260 nm. For Northern analysis, total RNA (10 μg/lane) was denatured with glyoxal, separated by size on 1.0% agarose gels and transferred to Genescreen (DuPont). The probe used was a 0.9 kb *BamH1* fragment (5' portion) derived from the full length human *nm23-H1.* cDNA probes were radiolabelled by nick translation system (Promega, Madison, USA) and hybridization. The membranes were washed twice in 2 x SSC and 0.1% SDS at 65˚C. The membranes were then exposed to Kodak Bio-Max MR film at 70˚C with intensifying screens.

Immunohistochemical localization of nm23 in gliomas. The paraffin-embedded sections of human normal brain tissue and gliomas were deparaffinized and incubated with the primary antibody (nm23-H1, diluted 1:200, Santa Cruz) for 15 min at 45˚C. Tissue sections for nm23 were incubated in Pepsin solution (Research Genetics, USA) for 3 min before application of the primary antibody. Biotin-labelled secondary antibody (GAR-HRP; Sigma, St. Louis, MO, USA) was utilized for 7 min at 45˚C. The streptavidin-horseradish peroxidase (Research Genetics) detection system was then applied to capillary channels, followed by 7 min of incubation at 45˚C. After drainage, the tissue sections were ready for chromogen reaction with 3-amino-9-ethy1 carazole (AEC). Counterstaining was performed with Harris-hematoxylin. As a negative control, secondary antibodies were used without the addition of primary antibodies.

Migration assay of primary cell culture. A migration assay in the early passage of the glioma cell culture was performed by a simple scratch technique. In order to eliminate any confounding effects of the experimental agents on cell proliferation, the media of the culture cells was replaced with medium containing 5 mM hydroxyurea. Twenty-four-h treatment with 5 mM hydroxyurea resulted in complete inhibition of cell proliferation. After 24 h of hydroxyurea treatment, the cultures were scraped with a single-edged razor blade. The cells were washed twice with phosphate-buffered saline (PBS) and placed in medium containing hydroxyurea. After 48 h of incubation, the cells were washed twice with PBS, fixed with absolute alcohol and stained with 0.1% toluidine blue. Three microscopic fields were evaluated for each wound injury. The number of cells migrating across the wound edge and the maximum distance migrated were determined in each

field and averaged for each injury. These experiments were repeated in triplicate. Statistical analyses were carried out by unpaired *t*-test.

Astrocytoma cell transfections and subcloning. Nm23-H1 cDNA was isolated by reverse transcription polymerase chain reaction (RT-PCR) using total RNA purified from human normal brain tissue. The primers for *nm23-H1* were as follows: sense primer, 5'-CGGAATTCCATGGCCAACTGTGAGCGT-3' (primer contained an EcoRI site) and antisense primer, 5'-CGGGATCCCGTCA TTCATAGATCCAGT -3' (primer contained a Bam HI site). The PCR products were cloned into the EcoRI-BamH1 site of the pEGFP-C1 vector (Clontech, CA, USA) and the nucleotide sequence was confirmed by automatic DNA sequencer ABI Prism 377 (PE Applied Bio-systems, USA). U87MG cells were transfected with 1 μg of *nm23-H1* expression plasmid or vector alone using the FuGENETM6 (Roche Molecular Biochemicals, Mannheim, Germany), according to the manufacturer's directions. G418 (500 μg/μl) (Gibco) was added to the cells 72 h later. G418-resistant clones were isolated and expanded in culture medium containing 200 μg/μl of G418. Approximately 24 individual *pEGFP-C1 nm23-H1*-transfected neomycin-resistant clones were isolated and characterized to identify high-expression clonal lines. Five lines were selected for the presence of an exogenous and endogenous nm23-H1 band on Western blots, expressing increased protein biosynthesis. Similarly, a clone of U87G transfected with pEGFP-C1 vector alone was used as control.

Doubling-time of stable transfectants. Transfectants with GFP and/or nm23-H1 and each parental cell were seeded on $5x10^5$ in 60-mm culture dishes. The cells were harvest and counted every 24 h after 48 h serum starvation. The doubling-time was calculated from the cell growth curve over 7 days.

Brain slice invasion study of transfectants. In an attempt to recapitulate the matrix macromolecule representation normally encountered by infiltrating astrocytoma cells, we adapted neural organotypic cultures, as previously described by Jung *et al.* (18, 19). By using the GFP-transfected astrocytoma cells, it was possible to evaluate the invasiveness in a brain slice model. Briefly, specimens of white matter were cut into 1-mm-thick, 8 x 8-mm^2 slices using a brain matrix (Harvard Instruments, Boston, MA, USA) and placed onto the upper chamber of 24-mm Transwell culture dishes (0.4 μm in pore size, Corning Costar Corporation, Cambridge, MA, USA). The brain slices were incubated in medium containing α-MEM including supplements such as glutamine (Gibco BRL), and 10% FCS, insulin-transferrin-splenium-A (100x) (Gibco BRL), glucose and 20 nM progesterone (Sigma), modified from that used by Tanaka *et al.* (3). $5x10^5$ astrocytoma cells were placed on a central hole of the brain slice and incubated for 10 days.

Tissue preparation and laser confocal microscopy analysis of invasion. After 10 days of culture, the whole brain slice was fixed with 4% paraformaldehyde at 4˚C overnight and rinsed carefully with PBS. The 1-mm-thick brain slice was placed on a microscopic slide and covered with mounting medium to maintain the 3-dimesional (3-D) structure. The sample was directly observed under fluorescent and confocal microscope. A 3-D image reconstruction (3-D Doctor) showed the invading spatial pattern of the tumor cells, much like the invasion found *in vivo*.

Results

Expression of nm23 in human gliomas. The immuno-histochemical study revealed variable degrees of *nm23-H1* expression in low- and high-grade gliomas (Figure 1). *Nm23* was notably localized in the cytoplasm. Although no significant correlations were detected between *nm23-H1* expression and pathological grade, *nm23-H1* expression in gliomas was distributed especially in the peripheral tumor margin (Figure 1C). The protein and RNA levels of nm23-H1 in gliomas were determined by Western blot, RT-PCR and Northern blot analysis. Each glioma demonstrated a distinct single band corresponding to 17.5 kDa, 435 bp and 0.8 kb, respectively, suggesting that there was no correlation with the histopathological grade (grade 1 to 4) in the protein and RNA levels of nm23-H1 (Figure 2).

Nm23 expression and migration in glioma primary cultures. On migration assay, the low-expression group (Group 2) of endogenous nm23-H1 in glioma primary culture cells showed further migration in greater numbers compared to the high-expression group (Group 1) (Figure 3). The maximum migration distance and mean cell number were 687 μm and 117 cells, respectively, for Group 1 and 927 μm and 208 cells for Group 2. The difference between the two groups was statistically significant in mean cell number ($p=0.0001$) and maximal distance ($p<0.0001$).

Isolation of stable GFP and nm23 transfectants. Stable high-level expression of GFP (U87G) or GFP-nm23 (U87G-nm23) transfectants were selected by direct visualization under fluorescent microscope and Western blot analysis for nm23-H1. The parental cell lines U87MG and U87G showed the same level of 17.5 kDa nm23, but the U87G-nm23 transfectant showed a faint band at 17.5 kDa and a marked strong band at 45 kDa, indicating the fusion protein of GFP and nm23 (Figure 4A).

Doubling-time. There was no significant difference in the cell proliferation rates among parental U87MG, selected U87G and U87G-nm23, with respect to their doubling-times. The doubling-times were 19, 23.6 and 24.71 h, respectively.

Nm23 transfectants and migration. The U87 astrocytoma cell line is an originally motile cell line. Following nm23-H1 transfection, U87G-nm23 became less motile in mean cell number and maximum migration distance, compared to both U87MG and U87G (Figures 4B and 5D-F). The maximum migration distance and mean cell number in the parental cell lines was 1315 μm and 363 cells for U87MG and 1404 μm and 409 cells for U87G. The migration profile of U87G-nm23 was 1089 μm/225 cells. The nm23-H1 transfectant showed an effect of approximately 45%

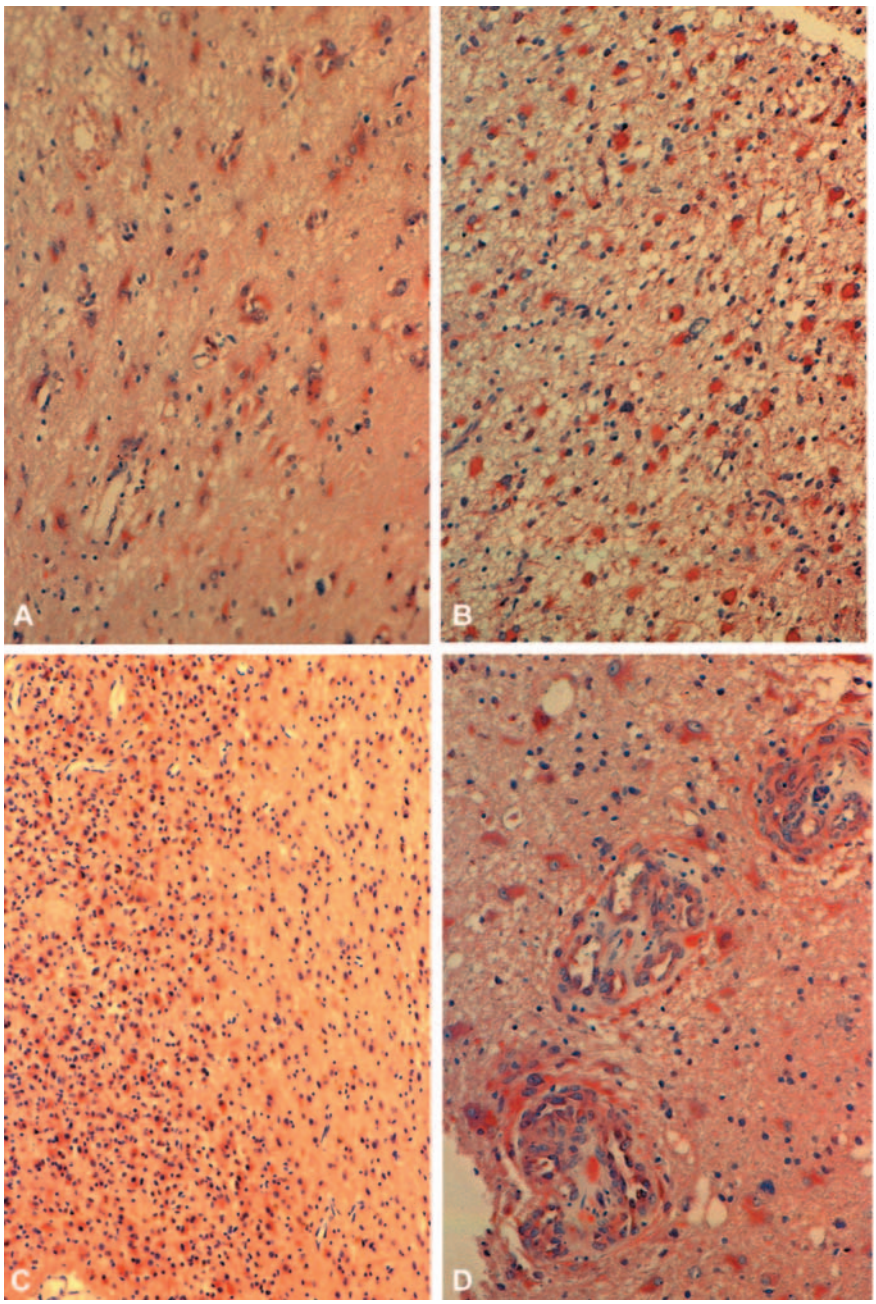

Figure 1. *Immunohistochemical study for nm23-H1 in gliomas. Human glioma cells demonstrated variable amounts of nm23-H1 expression in low- and high-grade gliomas (A; astrocytoma, B; anaplastic mixed glioma, C; anaplastic oligodendroglioma, D; glioblastoma).*

reduction of the parental migration rate. We also observed the morphological changes of the transfectant cell line compared to the parental cell lines. There were no significant changes in the shape and size of the cells following nm23-H1 transfection (Figure 5A-C).

Brain slice invasion study of transfectants. The invasiveness of *GFP*-transfected astrocytoma cells could be evaluated in the brain slice by the organotypic culture technique, described by Jung *et al.* (18, 19). U87G cells, transfected only with *GFP*, migrated widely throughout the surrounding normal brain (Figure 6A), but U87G-*nm23* cells, transfected with both of *GFP* and *nm23*, showed marked reduction of invasiveness of the parental control, U87G cells (Figure 6B). Most of these cells were found around the implanted site of the brain slice (Figure 6B).

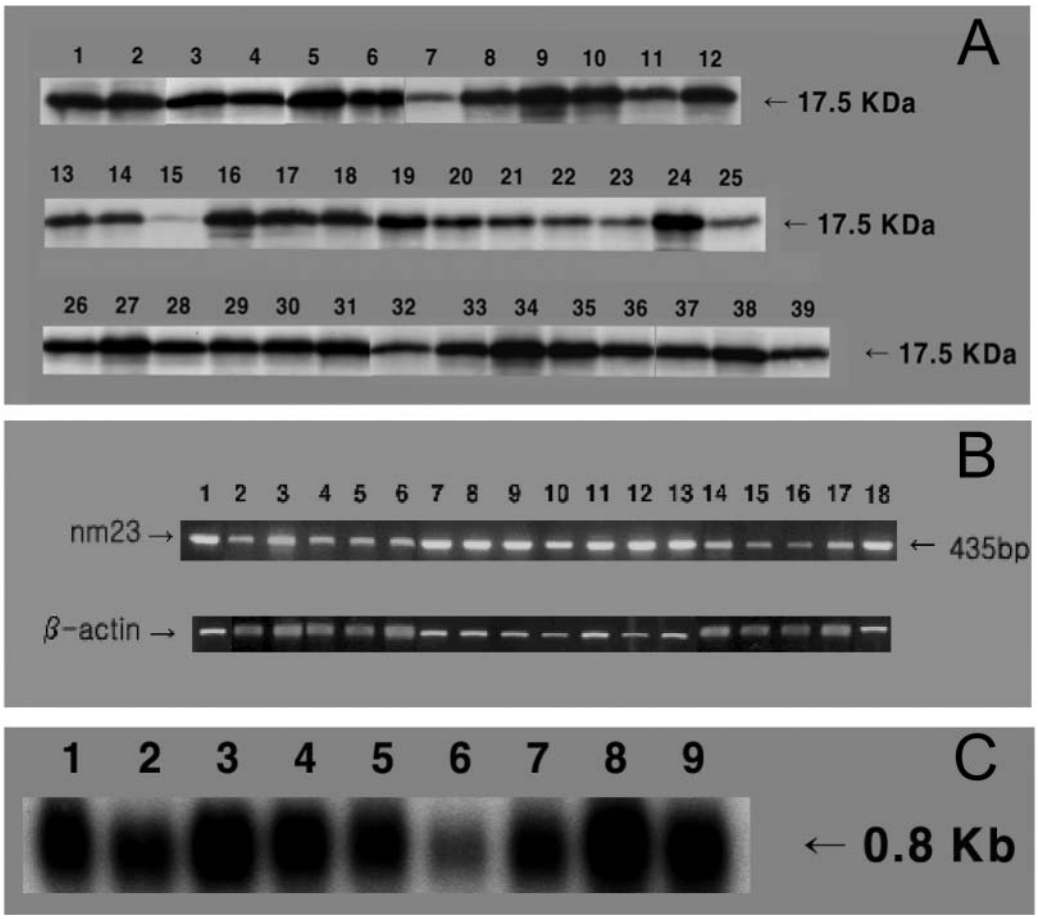

Figure 2. *The protein and RNA level of nm23-H1 in gliomas as determined by Western blot analysis (A), RT-PCR (B) and Northern blot analysis (C) according to the pathological grade. Each glioma showing distinct single bands corresponding to 17.5 kDa, 435 bp and 0.8 kb, respectively, suggesting that there was no correlation with the histological grade (Grade 1 to 4) in the nm23 expression (A: 1 and 2; normal brain, 3 and 4; Grade I astrocytoma, 5; Grade II astrocytoma, 6; Grade III astrocytoma, 7 ~ 25; glioblastoma, 26 ~ 28; oligodendroglioma, 29 ~ 34; anaplastic oligodendroglioma, 35 and 36; anaplastic ependymoma, 37 ~ 39; anaplastic mixed glioma, B: 1; Grade I astrocytoma, 2 ~ 13; glioblastoma, 14 and 15; Oligodendroglioma, 16 ~ 18; anaplastic oligodendroglioma, C: 1; Grade I astrocytoma, 2; anaplastic astrocytoma, 3 ~ 9; glioblastoma).*

Discussion

The metastastic process of cancer is a fundamental aspect of tumor progression and a major contributor to associated mortality. The insidious infiltration of brain tumor cells into the surrounding normal brain renders the complete and thorough removal of the tumor microscopically impossible and, therefore, represents a primary cause of therapeutic failure. The *nm23* gene was initially cloned as a metastasis suppressor gene, but the clinical relevance of *nm23* as a metastasis suppressor, or a prognostic indicator for human cancer, remains unknown. The *nm23* gene encodes nucleoside diphosphate (NDP) kinase, which exhibits a metastatic suppressor effect in some tumors, including breast carcinoma, hepatocellular carcinoma, ovarian carcinoma, bladder carcinoma, melanoma, lung carcinoma, colon carcinoma and pheochromocytoma (5, 7-11, 20-23). However, in other tumors, including neuroblastoma and pancreatic carcinoma, this metastatic suppressor effect is not detected (24-26). *Nm23* has been characterized as a gene family, and is a multi-functional protein, with well-documented enzymatic and DNA-binding functions (27). Five isoforms of *nm23* exist, of which two, *nm23-H1* and *nm23-H2*, have been widely studied within the context of metastatic suppression. The genes of these two isoforms are localized on chromosome 17q21, and the others are located on chromosome 16 (28, 29). Biological factors, including cell motility and migration, changes in adhesion molecules and proteolytic enzymes, are all quite relevant to metastatic and locally invasive cells. The metastatic suppressive effects of

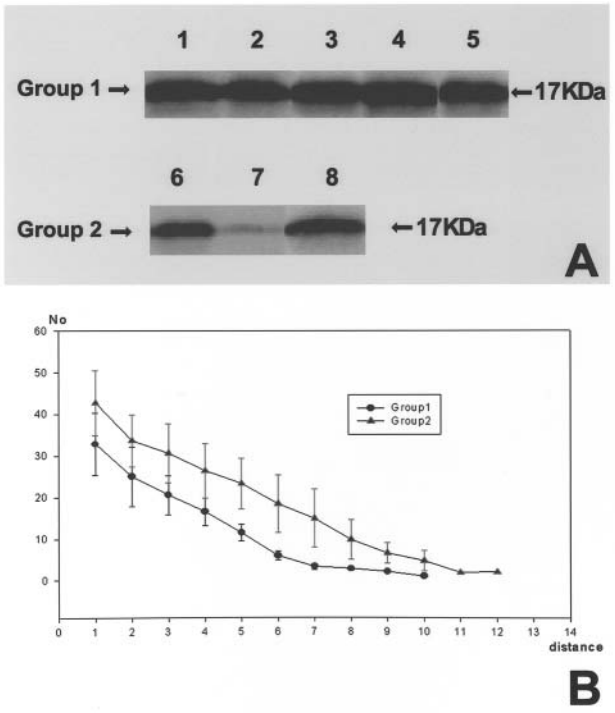

Figure 3. *Correlation between migration ability and nm23-H1 expression of glioma primary culture cells. (A) Western blot analysis demonstrating Group 1 with high expression of nm23-H1 and Group 2 with low expression. (B) Comparison of the migration ability using in vitro assay between Groups 1 and 2. Group 2 showed further migration in greater numbers with statistical significance, when compared to the results for Group 1.*

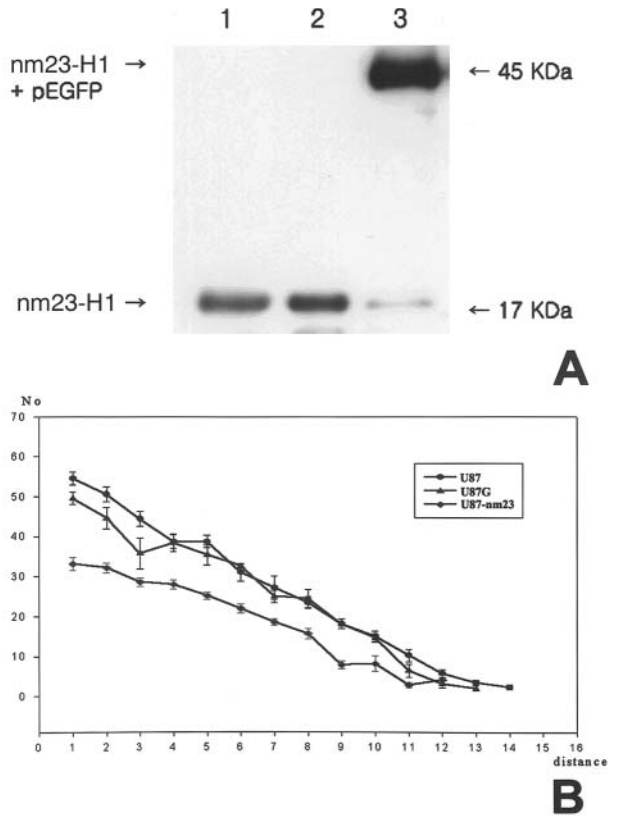

Figure 4. *Correlation between migration ability and nm23-H1 expression in parental cells and transfectants. Western blot analysis (A) showing different expression of nm23-H1 in parental cells and transfectants (1; parental U87MG (U87), 2; GFP-transfectant (U87G), 3; GFP-nm23-transfectant (U87G-nm23)). Comparison of the migration ability (B) using in vitro assay between parental cells and nm23-H1 transfectants. The nm23-H1 transfectant shows approximately 45% reduction of the parental migration.*

nm23 have been attributed to microtubule-associated NDP kinase (NDPK) activity (30, 31), serine phosphorylation (32, 33), signal transduction (34, 35), or its DNA binding capability (29, 36, 37). NDPK has been identified as a microtubule-associated protein, and has been loosely associated with microtubule assembly and disassembly and may, therefore, influence the cytoskeletal status. There is growing evidence that *nm23* inhibits cell migratory ability in response to micro-environmental factors (38, 39).

Despite the well-documented importance of *nm23* in the control of metastasis, there is a paucity of data concerning the role of this gene family in the control of brain tumor invasion. Meningiomas of different pathological grade have been tested, and *nm23-H1* expression was determined to be significantly higher in those tumors, during the advanced stages of tumor progression (40). In astrocytic tumors, high nm23 expression levels might be correlated with extraneural metastatic potential (41). However, levels of *nm23* expression appear to be inversely related to cavernous sinus invasion, suggesting the role of *nm23* in the progression of invasive pituitary tumors (42). The aim of this study was to investigate *nm23-H1* expressions in human gliomas, and to assess the effects of transfection with the metastatic suppressor gene, *nm23-H1*. In the present study, human glioma cells evidenced variable amounts of *nm23-H1* expression in low- and high-grade gliomas. Although no significant correlations were detected between *nm23-H1* expression and pathological grade, *nm23-H1* expression in gliomas was distributed especially in the peripheral tumor margin. Moreover, the migratory ability of the primary culture cells was found to be inversely correlated with *nm23-H1* expression, as shown in the *in vitro* migration assay in our study.

It is well known that low-grade, well-differentiated gliomas also exhibit extensive infiltration into the surrounding brain tissue. In this respect, gliomas differ from other systemic metastatic tumors, in which invasion and metastasis usually occur at later stages in the progression of the disease. Regardless of pathological grade, each glioma cell may exhibit a peculiar invasiveness. The effect of the nm23-H1 transfectant on the migratory and invasive abilities of human glioma cell lines was evaluated *via in vitro* assay, a simple scratch technique,

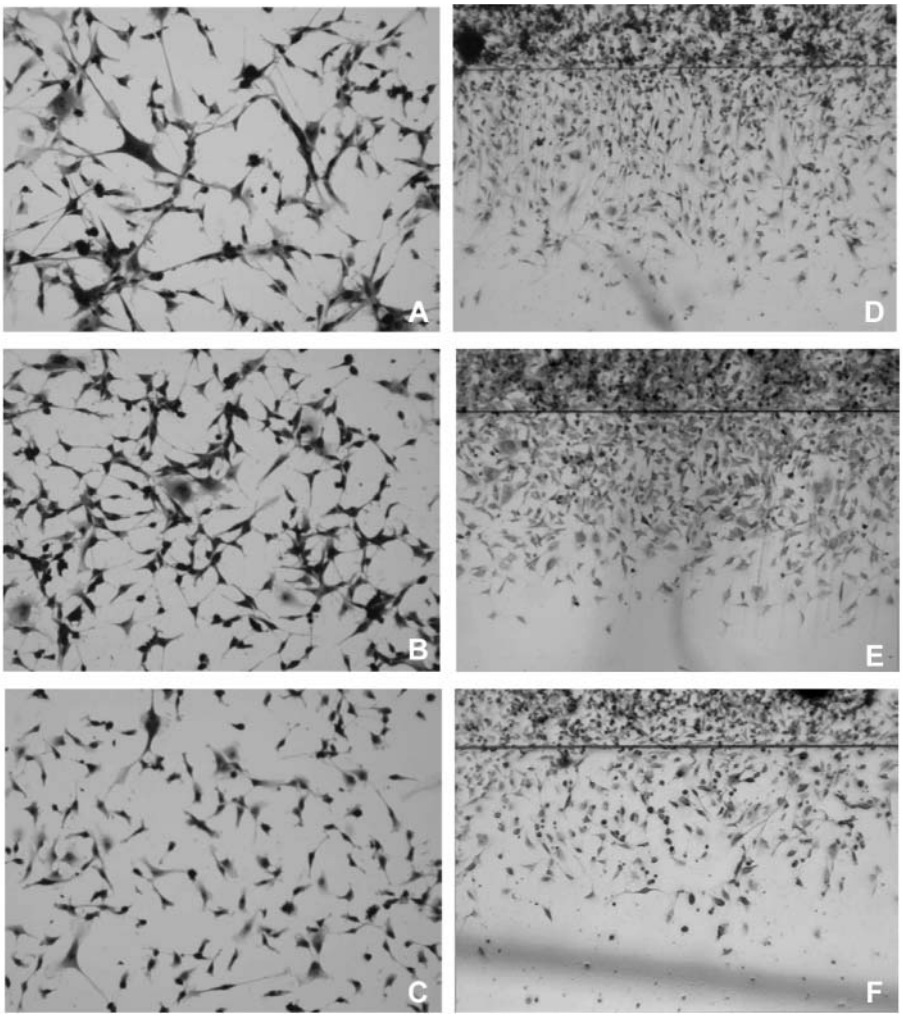

Figure 5. *Photomicrographs showing cell morphology (A-C) and migration status (D-F) (A and D; parental U87MG, B and E; GFP-transfectant (U87G), C and F; GFP and nm23-H1 transfectant (U87G-nm23)). Note that U87G-nm23 cells showed lower motility in cell number and migrated distance than the parental U87MG and U87G lines. There were no significant changes in the shape and size of the cells following nm23 transfection.*

and the brain slice model system. A variety of invasion model systems for *in vivo* and *in vitro* studies have been introduced in the study of brain tumor invasion, and the selection of a proper model system is of inestimable importance in the evaluation of migratory and invasive potential.

Transplanted tumors in animal models progress principally by expansion, rather than by diffuse filtration, as a result of their high rates of proliferation (43). The simple scratch technique in a confluent cell monolayer (44) and, together with the Matrigel method (45), are rapid, quantitative methods, with many factors influencing invasion having been identified, although their relevance has occasionally been called into question. With regard to a suitable matrix for the tumor, organotypic coculture models have been used to study the invasiveness of brain tumors (18, 19, 46, 47). Living brain tissue, especially human brain tissue, is an ideal matrix in which to study malignant glioma cell invasion *in vitro*. In experimental tumor models and histopathological preparations, single-cell infiltration and micrometastasis have often proven difficult to study, due to a lack of suitable and sensitive markers which can discriminate individual tumor cells from the normal cell population. The green fluorescent protein (GFP) has been used to monitor gene expression and protein localization in living organisms, as well as in the visualization of cancer invasion and metastasis (18, 48). The study of brain tumor invasion on a 3-D brain slice model system has proven more vital and reliable than other *in vitro* models.

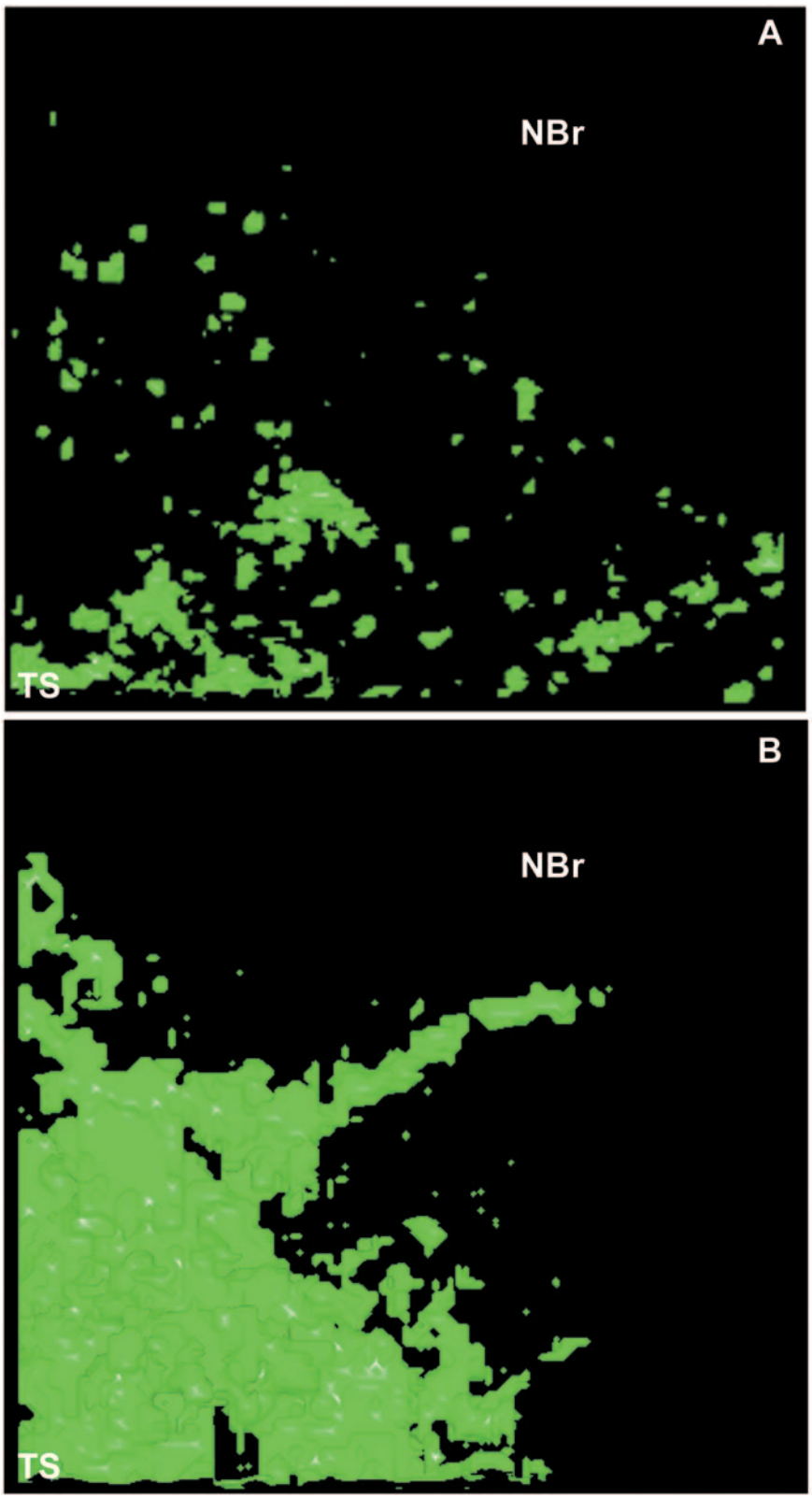

Figure 6. *Organotypic cultures showing invasion of transfectants through the surrounding normal brain. (A) U87G cells showed extensive migration of the tumor cells throughout the surrounding brain tissue. (B) U87G-nm23 cells demonstrated marked reduction of invasiveness of the parental control, U87G cells, and remained near the implanted site. (TS; implanted tumor spheroid, NBr; normal brain (dark background)).*

In the present study, the simple scratch technique was utilized for the comparison of migratory ability in the primary culture cells, and in the transfectant human glioma cell lines. *GFP*-gene transfected human glioma cells (U87G) were used to visualize the progression of tumors through the human brain *in vitro*, in order to compare with the invasiveness of the nm23-H1 transfectant cells (U87G-nm23). The migratory ability of the U87G-nm23 was reduced by approximately 45%, as compared to the parental cell line. We also observed a unique effect of nm23 in the reduction of glioma invasion. Most of the nm23-H1 transfectant cells maintained positioning around the original implantation hole, while the parental cells, which express only *GFP*, migrated widely throughout the brain. Gliomas expressed variable levels of nm23-H1, and the expression of nm23-H1 in gliomas was inversely correlated with their migratory ability in an *in vitro* assay, although there was no correlation between nm23-H1 expression and pathological grade. The basal migratory ability of the nm23-H1 transfectants was less pronounced than that of the parental cell lines, and the invasive properties of the nm23-H1 transfectants were markedly reduced in the brain slice model system, as compared to that of the parental cells. Overall, these findings suggest that *nm23-H1* may play an important role in the suppression of human glioma invasion and migration.

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