REVIEW ARTICLE

Introduction

Most cancers acquire six functional capabilities during their development, including self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis [1]. The order in which these capabilities are acquired seems to be quite variable across the spectrum of cancer types and subtypes. Importantly, the acquisition of metastatic ability leads to clinically incurable disease for most cancer cell types [2]. Cancer metastasis is a critical factor in the prognosis of cancer patients and is considered to be a significant contributor to mortality. Improvements in surgery and radiotherapy and the development of new chemotherapeutic agents or their use in new combinations have, so far, only incrementally improved patient survival. Despite the metastatic process remaining incompletely characterized at the molecular and biochemical levels [3], a novel class of genes—metastasis suppressors—identify new facets of the process and are important therapeutic targets. The first metastasis suppressor, nm23, was identified in 1988 [4], and since then eight suppressor genes have been confirmed [2]. These suppressor genes comprise NM23, MKK4, KA11, BRMS1, KSS1, RHOGD12, CRSP3, and VDUP1. Because many studies have correlated the nm23 gene

NM23-H1: A METASTASIS-ASSOCIATED GENE

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SUMMARY

The protein product of nm23-H1 gene has activity of nucleoside diphosphate (NDP) kinase, which catalyzes the phosphorylation of nucleoside diphosphates to the corresponding nucleoside triphosphates. Reductions in nm23 expression have been significantly associated with aggressive behavior in melanoma, breast, colon, and gastric carcinomas. On the contrary, high levels of nm23 gene expression are noted in the advanced stage of thyroid carcinomas and associated with significant reductions in survival for neuroblastoma and osteosarcoma patients. Although expression of nm23/NDP kinase is divergent in various malignant tumors, its reduced expression seems to be related to increased metastatic potential in most carcinoma types. However, it is hypothesized that nm23 may play a tissue-specific role, and that different regulatory mechanisms may act in different tumors. In ovarian carcinoma, nm23-H1/NDP kinase may be correlated with some clinicopathologic characteristics. In cervical cancer, nm23-H1 is probably involved in cervical carcinogenesis and correlated with some aggressive parameters. Overexpression of nm23-H1 protein may indicate poor survival for cervical cancer patients. Other than histidine 118 residue (amino acid sequence 118: histidine) concerned with NDP kinase activity of nm23-H1, serine 120 (amino acid sequence 120: serine) related activity of histidine-dependent protein phosphotransfer was recently reported to be responsible for its biological suppressive effects. To inhibit metastatic potential, nm23-H1 is also demonstrated to co-immunoprecipitate the kinase suppressor of Ras and phosphorylate it, and therefore reduce activation of the extracellular signal-regulated kinase mitogen-activated protein kinase pathway in response to signaling. [Taiwanese J Obstet Gynecol 2006;45(2):107–113]

Key Words: high-grade squamous intraepithelial lesion, kinase suppressor of Ras, metastasis, Nm23-H1, nucleoside diphosphate kinase, squamous cell carcinoma

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with metastasis and prognosis for gynecologic cancers, it is very important to recognize this gene.

Discovery of Nm23/NDP Kinases

Nucleoside diphosphate (NDP) kinase was first discovered in yeast [5] and in pigeon breast muscle [6]. In human erythrocytes and other mammalian tissues, electrophoretic profiles were complex, suggesting that several isozymes were present [7,8]. The first primary structures for NDP kinases were reported in 1990 for _Myxoccus xanthus_ [9], _Dictyostelium discoideum_ [10], and rat [11]. This led to the discovery that the product of an independently isolated regulatory gene is NDP kinase [12]. The first _nm23_ gene was isolated by Steeg et al in 1989, on the basis of its reduced expression in highly metastatic murine meloma cell lines, as compared with their nonmetastatic counterparts, and has been proposed as a metastatic suppressor gene [4]. They utilized differential colony hybridization to analyze seven cell lines derived from a murine K-1735 melanoma with varying metastatic potential. They found that clone 23, exhibiting the highest RNA levels, was associated with lower metastatic potential. Therefore, this gene was termed as nonmetastatic clone 23, which was abbreviated to _nm23_. The second _nm23_ gene of mouse was subsequently found by Urano et al and termed _nm23-M2_ [13]. Then the gene, which was found by Steeg et al, was regarded as _nm23-M1_. In 1989, the first human equivalent was isolated by screening a human fibroblast cDNA library [14]. Rosengard et al identified the _nm23_ gene, for which RNA levels were reduced in tumor cells of high metastatic potential. This gene was referred to as _nm23-H1_. The human Nm23 protein has sequence homology over the entire translated region with a described developmentally regulated protein in _Drosophila_, encoded by the _awd_ gene. Thus far, at least eight genes ( _nm23-H1_ to _nm23-H8_ ) have been documented [15].

**Human Nm23/NDP Kinases Family**

A second human gene, _nm23-H2_, was identified by its high homology to _nm23-H1_ [16,17]. The _nm23-H1_ and _nm23-H2_ gene products share 88% identity.

The protein products of _nm23-H1_ and _nm23-H2_ are identical to human NDP kinase A and NDP kinase B, separately. The _nm23-H3_ gene (also known as DR-nm23) was identified by differential screening of a cDNA library obtained from chronic myelogenous leukemia cells [18]. The remaining five members of the _nm23_ gene, named _nm23-H4_ to _nm23-H8_, were identified by searching for homologous sequences in the expressed sequence tags database [19–22], although, as far as we know, they do not affect metastatic potential. Lacombe et al found that eight human genes of the _nm23/NDP kinase_ family can be separated into two groups based on the analysis of their sequences [15]. The group I ( _Nm23-H1_ to -H4_ ) genes encode proteins, which possess the classic enzymatic activity of an NDP kinase. This group includes NDP kinases A–D, which share 58–88% identity with each other. The protein products of the group II genes ( _Nm23-H5_ to -H8_ ) are more divergent as the sequences share only 25–45% identity with the first group proteins and between each other.

However, only one product of group II genes ( _Nm23-H6_ ) has been demonstrated to catalyze the NDP kinase reaction.

Functions of Human Nm23/NDP Kinases

**Nucleoside diphosphate kinase activity**

The _nm23-H1_ gene product has been proposed to be an NDP kinase on the basis of its homology with the _Dictyostelium discoideum_ enzyme [10,12]. It has been further identified as the human NDP kinase A isoform purified from erythrocytes, whereas the _nm23-H2_ gene product has been identified as the NDP kinase B isoform [17]. The NDP kinases are 17–20 kDa proteins that are distributed ubiquitously and catalyze the phosphorylation of nucleoside diphosphates to the corresponding nucleoside triphosphates, mainly at the expense of the ATP synthesized through oxidative phosphorylation [23]. It can be expressed by a formula:

\[
N_1TP + N_2DP \xrightarrow{\text{NDP kinase}} N_1DP + N_2TP
\]

The enzymatic activity involves the transfer of the γ-phosphate of nucleoside triphosphate (NTP) to NDP, where _N_1 and _N_2 can be ribo- or deoxyribonucleosides, via a high-energy _Nm23-phosphohistidine_ intermediate [24]. The mechanism also involves a conserved histidine, namely histidine 118 (amino acid sequence 118: histidine) in the human enzymes [17], as a phosphorylated intermediate with a high-energy bond ~7 kcal/mole [24,25].

**Metabolic roles of mammalian NDP kinases**

Until now, the widely accepted roles of NDP kinases are: (1) to use ATP as a donor to synthesize the non-adenylic NTPs needed for nucleic acid synthesis and
several important metabolic intermediates including UDP-glucose and a few CDP-lipid derivatives; (2) to catalyze transphosphorylation between GTP produced in the Krebs cycle and ADP; and (3) to provide GTP for protein synthesis, G-protein signaling, and tubulin polymerization [15]. Nonadenylic nucleotides, especially GTP, play a number of key metabolic and regulatory roles in the cell. Furthermore, the ratio of [GTP]/[GDP] and [ATP]/[ADP] can be maintained differently if they are generated by different metabolic processes and if NDP kinase is prevented from freely equilibrating the guanine and adenine nucleotide pools.

**Cellular response to nm23-H1**

In our laboratory, we found that different cervical cancer cells exerted different Nm23-H1 protein levels. Excessive Nm23-H1 protein content were found in HeLa cells using Western blotting. On the contrary, in Caski cells, no Nm23-H1 protein was detected. The amount of Nm23-H1 protein detected in SiHa cells ranged between that in HeLa and Caski cells. Using matrix zymography (it is applied to detect the function of matrix metalloproteinase), Caski cells (no Nm23-H1 protein content) were found to exhibit the highest proteinase function, which is related to degradation of extracellular matrix and then invasion. In contrast, HeLa cells (excessive Nm23-H1 protein content) present little proteinase function. Therefore, in cellular levels, nm23-H1 seems to inhibit the metastatic potential of cervical cancer cells. The interesting cellular response is also demonstrated in different cancer cell types. In melanoma and breast carcinoma cells overexpressing nm23-H1, a reduced response to the cytokine TFG-β1 was found [26,27]. Furthermore, Kantor et al also found that their motility in response to serum and platelet derived growth factor is markedly inhibited [28]. In addition, medroxyprogesterone acetate appears to elevate Nm23-H1 metastasis suppressor gene expression, thereby reducing metastatic colonization in metastatic human breast carcinoma cells [29,30].

In the L9981 lung cancer cell line, the nm23-H1 gene can reverse the malignant and metastatic phenotype of cancer cells through regulating the expression of lung cancer metastasis-related genes [31,32]. In the DU 145 prostate cancer cell line, the nm23-H1 gene product suppresses metastatic potential by inhibiting ability of cancer cells in anchorage-independent growth and extracellular matrix adhesion [33].

Although histidine 118 (amino acid sequence 118: histidine) is involved in Nm23's NDP kinase activity, MacDonald et al found that mainly serine 120 (amino acid sequence 120: serine), instead of histidine 118, may be contributory to its tumor metastasis-suppressive capacity [34]. They utilized site-directed mutagenesis for proline 96 or serine 120 of nm23-H1 in MDA-MB-435 human breast carcinoma cells to abrogate its motility inhibitory activity in breast cancer cells. MacDonald et al also showed that Nm23 serine phosphorylation cannot directly participate in the NDP kinase activity of Nm23 because of its low bond energy. Lee and Lee further indicated that alterations at the histidine 118 residue, which result in loss of NDP kinase activity of nm23-H1, do not affect nm23-H1's inhibitory activity on the colonogenicity of DU 145 prostate carcinoma cells [35]. They suggested that the metastasis suppressing function of nm23-H1 is independent of the NDP kinase enzymatic activity. Freije et al found that the Nm23-H1 (S120A) mutant (amino acid sequence 120: serine is mutated to alanine) was deficient in only histidine-dependent serine autophosphorylation [36]. Nm23-H1 and Nm23-H1 (S44A) mutant (amino acid sequence 44: serine is mutated to alanine) exhibited normal activity in all assays conducted. Based on this correlation, they hypothesized that a histidine-dependent protein phosphotransfer activity of Nm23-H1 may be responsible for the biological suppressive effects of nm23-H1.

The metastasis suppressive function of nm23 was previously correlated with its histidine protein-kinase activity in a site-directed mutagenesis experiment [36]. Furthermore, Nm23 is reported to co-immunoprecipitate the kinase suppressor of Ras (KSR) protein—a scaffold protein for the ERK-MAPK (extracellular signal-regulated kinase mitogen-activated protein kinase) pathway and phosphorylate KSR serine; further alter its scaffold function and could lead to reduced ERK activation in response to signaling [37–45].

In contrast, Valentijn et al found that N-myc upregulates expression of nm23-H1 gene in neuroblastoma cells and increases Nm23-H1 protein levels. Thereafter, Nm23-H1 protein binds to cell division cell 42 and prevents the induction of differentiation [46]. This may increase the metastatic potential of neuroblastoma cells. In brief, nm23 may play a tissue-specific role, and influence the metastatic behavior of different cancer cells via different regulatory mechanisms. An inverse relationship, however, exists between Nm23-H1 protein and metastatic potential in most cancer cell types except in rare cancer cell types (such as neuroblastoma cells).

Interestingly, Nm23/NDP kinase is further demonstrated to link cytoskeletal components, tubulin and vimentin, which are involved in the spatial features of cell signaling [47–50]. Activation of transmembrane receptors often affects cytoskeletal organization and the association of Nm23/NDP kinase with structural proteins, such as vimentin, could affect the dynamics
of cytoskeleton remodeling following external stimulation. Vimentin networks show a tendency to form tangles and bundles and can be more densely packed when assembled in the presence of Nm23/NDP kinase, presumably because more than one filament can interact with the Nm23/NDP kinase hexamer. Overexpression of Nm23/NDP kinase could reduce the flexibility necessary for cytoskeleton plasticity and cell movement and this property could be the basis for the motility suppressive actions [51,52]. Thereafter, tissue invasion of cancer cells is inhibited.

**Nm23-H1 Expression in Various Tumors**

Nm23-H1 has been regarded as metastasis-associated genes in various tumors [15,53]. Reductions in nm23 expression have been significantly associated with aggressive behavior in melanoma, gastric, colon, and breast carcinoma. Expression of nm23-H1 is inversely proportional to the metastasis of these carcinomas [54–57]. On the contrary, high levels of nm23 gene expression are noted in the advanced stage of thyroid carcinomas [58,59]. In addition, elevated nm23-H1 expression is associated with a significant reduction in survival for neuroblastoma and osteosarcoma patients [60,61]. However, Szumilo et al suggest that nm23 status is not associated with metastatic ability and prognosis in esophageal squamous cell carcinoma (SCC) [62]. Obviously, nm23 may play a tissue-specific role, and different regulatory mechanisms may act in different tumors.

**Nm23-H1 Expression in Gynecologic Tumors**

**Nm23-H1 and ovarian cancer**

Mandai et al demonstrated that low levels of nm23-H1 expression have been associated with lymph node metastasis and advanced stage in ovarian carcinoma [63]. Tas et al found that the nm23 staining was more intensive in patients with normal serum CA19.9 levels, patients with nonrecurrent disease, and alive patients (p < 0.05). However, they found that Nm23 expression was not correlated with common clinicopathologic parameters such as histology, grade of differentiation, International Federation of Gynecology and Obstetrics stage, and CA-125 [64]. They also demonstrated that nm23 may have a favorable prognostic impact on ovarian cancer. In contrast, Tomic et al reported that the overexpression of nm23 proteins was associated with advanced clinical stage, high grade, and/or presence of vascular invasion for epithelial ovarian carcinoma [65]. They found that this overexpression, however, had no independent prognostic value either for overall survival or survival corrected by clinical stages. Therefore, the role of nm23-H1 in ovarian carcinoma remains unclear.

**Nm23-H1 and cervical cancer and dysplasia**

Marone et al demonstrated that cervical cancer patients with lymph node involvement have significantly lower protein levels of nm23-H1 [66]. But they did not mention the histologic type of cervical cancer. Moreover, negative expression of nm23-H1 in cervical cancer has been found to be associated with a high incidence of lymph node metastasis in adenocarcinoma, but not in SCC [67]. However, Kristensen et al did not find immunostaining for nm23 to be a useful prognostic indicator in cancer of the uterine cervix [68]. Our previous study correlated nm23-H1 immunoreactivity with clinicopathologic variables; a trend was determined for high nm23-H1 expression for older women and metastatic status, however, statistical significance was not achieved. Further, a significant relationship with high nm23 expression was only demonstrated for deep stromal invasion (≥1/2 stromal invasion depth) [69]. In addition to parametrial involvement, grade and lymph node metastasis, Sarac et al also did not find a significant association between nm23 expression and deep stromal invasion [70]. They did not, however, define the depth of stromal invasion. It seems reasonable to suggest that the relationship between deep stromal invasion and high nm23 expression is attributable to nm23/NDP kinase-related cellular proliferation. Furthermore, we also found that high cumulative recurrence hazard was demonstrated in the high nm23 expression subgroup of cervical cancer [69]. Our findings were in accordance with those of Chen et al, who found a significant association between lower recurrence-free survival rate (higher recurrence) and nm23 overexpression [71].

Many investigators have correlated nm23-H1 expression with metastatic potential, but few have studied its role in tumor progression. In another study, we found significant differences in the levels of nm23-H1 expression between low-grade squamous intraepithelial lesion (LSIL) and high-grade squamous intraepithelial lesion (HSIL) and between LSIL and SCC, but not between HSIL and SCC or normal and LSIL samples [72]. Furthermore, a positive relationship was demonstrated for high nm23-H1 protein expression and degree of malignant transformation. High nm23-H1 expression appears to be related to critical progression of LSIL to HSIL in cervical carcinogenesis. These findings are
consistent with the findings of Ravazoula et al, who demonstrated general trends of increasing immunoreactivity of nm23-H1 protein and progressively greater atypia in cervical epithelium [73]. Our findings are further supported by Weber et al and Ohneda et al [74,75]. Weber et al showed that Nm23/NPD kinases are crucial for the supplement of nucleoside triphosphates required for nucleic acid synthesis. Thus, an increased expression of this enzyme is linked to cellular proliferation. Ohneda et al suggested that cell transformation and immortalization in the early stage of tumorigenesis could induce nm23 gene overexpression, resulting in increased expression of nm23 gene product. Therefore, the nm23-H1 gene is not only involved in cervical carcinogenesis but also plays a critical role.

Conclusively, a high level of expression of the nm23-H1 gene product is found in most HSIL and SCC cases. High expression of Nm23-H1 protein indicates a critical progression from LSIL to HSIL involved in the subsequent emergence of invasive SCC. Ascending degree of malignancy transformation of cervical squamous neoplasia is reflected in the trend of increasing levels of nm23-H1 gene product expression. Because some conflicting findings still exist between the expression of nm23/NPD kinase and clinicopathologic characteristics in many studies of cervical cancer, we still cannot draw a conclusion concerning nm23-H1 with metastasis. However, overexpression of Nm23-H1 protein seems to predict a poor survival for cervical cancer patients.

Conclusion

Nm23-H1/NPD kinase is associated with tumor metastasis. Although its expression is divergent in various malignant tumors, its reduced expression seems to be related to increased metastatic potential in most cancer cell types. In ovarian carcinoma, the relationships of nm23-H1/NPD kinase and clinicopathologic characteristics still remain to be clarified. In cervical cancer, in addition to patients’ survival, nm23-H1 may also be involved in cervical carcinogenesis. Recent studies showing that Nm23 co-immunoprecipitates the KSR protein and, therefore, reduces ERK-MARK activation in response to signaling are interesting and warrant further research.

References


