

Regulatory functions of Nm23-H2 in tumorigenesis: insights from biochemical to clinical perspectives

Yuanjun Li · Yao Tong · Yung H. Wong

Received: 8 May 2014 / Accepted: 7 November 2014 / Published online: 21 November 2014
© Springer-Verlag Berlin Heidelberg 2014

Abstract Substantial effort has been directed at elucidating the functions of the products of the Nm23 tumor metastasis suppressor genes over the past two decades, with the ultimate goal of exploring their translational potentials in changing cancer patients' outcomes. Much attention has been focused on the better-known Nm23-H1, but despite having high sequence similarity, Nm23-H2 functions differently in many aspects. Besides acting as a metastasis suppressor, compelling data suggest that Nm23-H2 may modulate various tumor-associated biological events to enhance tumorigenesis in human solid tumors and hematological malignancies. Linkage to tumorigenesis may occur through the ability of Nm23-H2 to regulate transcription, cell proliferation, apoptosis, differentiation, and telomerase activity. In this review, we examine the linkages of Nm23-H2 to tumorigenesis in terms of its biochemical and structural properties and discuss its potential role in various tumor-associated events.

Keywords Cancer · Nm23-H2 · Tumorigenesis · Protein interaction

Introduction

The human Nm23 (non-metastatic 23, also known as NME) is a family of genes that are involved in diverse physiological and pathological processes ranging from cell proliferation, differentiation, development, and ciliary functions, to

metastasis (Steeg 1991; Boissan et al. 2009). Ten Nm23 homologues (H1–H10) have been discovered in humans thus far and they are classified into two major groups by phylogenetic analysis (Lacombe et al. 2000). Among the different members, Nm23-H1 and Nm23-H2 have been extensively studied in the context of cancer metastasis. Both Nm23 isoforms exhibit similar level of NDPK activity and they can also act as nucleases. However, observations from murine melanoma metastasis suggest a dissociation of the NDPK activity from the anti-metastasis phenotype (MacDonald et al. 1993). Nm23-H1 is regarded as the prototypical metastasis suppressor ever since the demonstration of its ability to reduce metastasis in a variety of cancer models (Steeg et al. 1988, 1993).

Like Nm23-H1, Nm23-H2 can also act as a metastasis suppressor (Arnaud-Dabernat et al. 2003). However, compared to the numerous studies emphasizing on anti-metastasis function, it has often been overlooked that expression of Nm23-H2 is positively correlated to primary tumor incidence in a number of cancer types (Lee et al. 2012; Iizuka et al. 2006; Manavi et al. 2007). Nm23-H2 was firstly identified as a transcription factor that may activate the c-Myc proto-oncogene (Postel et al. 1993), but this view has been challenged (Steeg et al. 2011). Nevertheless, overexpression of Nm23-H2 in hepatocellular carcinoma cell lines results in an upregulation of c-Myc and tumor formation in athymic mice (Lee et al. 2012). Although the inverse relationship between the expression of Nm23-H2 and metastasis is generally accepted, its role in regulating tumorigenesis remains unresolved. In this review, we will examine the role of Nm23-H2 in tumorigenesis of different types of human tumors with regard to its regulation of gene expression, protein-protein interaction, signaling pathways, cellular proliferation, and differentiation. Although Nm23-H1/2 proteins share 88 % sequence identity, they have more distinct interacting partners than common ones. Functional multiplicity of Nm23-H2 will be described in

Y. Li · Y. Tong · Y. H. Wong
Division of Life Science and the Biotechnology Research Institute,
Hong Kong University of Science and Technology, Clear Water Bay,
Kowloon, Hong Kong, China

Y. H. Wong (✉)
State Key Laboratory of Molecular Neuroscience, Hong Kong
University of Science and Technology, Clear Water Bay, Kowloon,
Hong Kong, China
e-mail: boyung@ust.hk

the context of its interaction with different signaling molecules that are linked to tumorigenesis.

Structural features and localization of Nm23-H2

Within the Nm23 family, the most studied isoforms Nm23-H1 and Nm23-H2 share 88 % identity in their primary structures. Like Nm23-H1, the structure of Nm23-H2 is rather simple with six α -helices partially covering a four-stranded antiparallel β -sheet (Fig. 1a; Webb et al. 1995; Chen et al. 2003). It is rather surprising that these small 17-kDa proteins are capable of forming homohexamers as well as heterohexamers at all ratios of H1 to H2 (Gilles et al. 1991; Urano et al. 1992). Formation of hexamers would allow Nm23-H1/2 to create new surface areas for protein-protein interaction, and this might explain the diverse range of associated proteins. One unresolved issue pertaining to tumor-related biological functions by Nm23-H2 is whether hexamer formation is an absolute requirement. The hexameric structure of Nm23-H2 is typically viewed as a trimer of dimeric subunits (Fig. 1b). The residues forming the interface within (V16, V21, G22, E23, K26, E29, L/M38 K39, and F40; Fig. 1c) and between dimers (L81, V89, P101, G106, and I110) are identical in Nm23-H1 and Nm23-H2, with one exception of a conserved substitution at position 38. Early mutagenesis studies of several of the subunit contact sites on Nm23-H1 (K26G/Q, K39A, and P101S/G) indicate that point mutations may not be sufficient to effectively prevent hexamer formation (Freije et al. 1997; Postel et al. 1996). It is interesting that Nm23-H1 and Nm23-H2 differ by only 18 amino acids and, excluding the two residues that form part of the multimer contact sites,

they are all located on exposed surfaces of the hexamer (Fig. 1d). As a result, the surface nature of Nm23-H2 is basic in contrast to the acidic character of Nm23-H1 (Webb et al. 1995), which may also explain the differences of their interacting partners and functions. Both Nm23-H1 and Nm23-H2 are mainly present in the cytoplasm, but they can also be found, at least transiently, associated to membranes and in nuclei. Interestingly, a recent study has shown that Nm23-H2 binds efficiently to liposomes via an anionic phospholipid partner, while Nm23-H1 may require a non-lipidic partner for membrane attachment (Francois-Moutal et al. 2014). This indicates that although these two isoforms share highly identical sequences and similar secondary structures, the 12 % structural difference is likely to constitute molecular determinants for interaction with specific protein partners. Given the ability of Nm23-H2 to form numerous complexes with other Nm23 isoforms as well as different partners that range from proteins, lipids, to DNA, elucidating the biological functions of Nm23-H2 represents one of the most complicated and demanding tasks imaginable.

Clinical evidence linking Nm23-H2 to tumorigenesis

Despite its similarity to Nm23-H1, the relationship between Nm23-H2 and tumorigenesis is far from clear. Sporadic investigations over the past two decades have examined Nm23-H2 from different perspectives, ranging from in vitro and in vivo experiments to clinical studies. The targeted tumor types varied from study to study with some arriving at relatively solid conclusions. It has been shown that Nm23-H2 is involved in several kinds of human cancers including

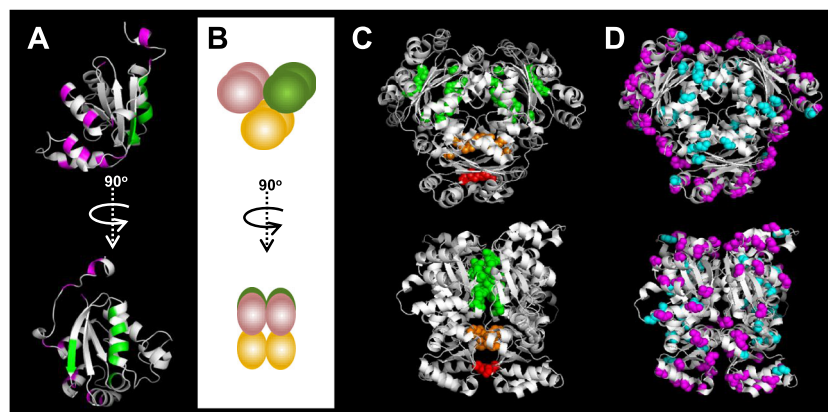


Fig. 1 Structural models of Nm23-H2 in monomeric and hexameric forms. The molecular modeling using Swiss-model and Pymol is based on a published Nm23-H2 crystal structure (PDB code: 1JXV). **a** Nm23-H2 monomer is shown in *white* with residues that are different from Nm23-H1 highlighted in *magenta*; residues involved in multimer binding are shown in *green*. **b** Schematic representation of the Nm23-H2 hexamer as the product of three pairs of dimers. **c** Residues involved in the

formation of the hexamer are shown as *space-filled spheres* in *green*, *orange*, or *red*; *orange* and *red* are used to highlight the two major clusters of interactive residues in the lower pair of dimers. **d** Structure of the Nm23-H2 hexamer with Nm23-H2-specific residues highlighted in *magenta* and as *space-filled spheres*; residues involved in nucleoside binding are highlighted in *cyan*. All Nm23-H2-specific residues are on exposed surfaces of the hexamer

hepatocarcinoma, endometrial, cervical, leukemia, breast, gastric, pleural mesothelioma, colorectal, lung, sarcoma, and giant cell tumor (Table 1).

Perhaps the most compelling evidence revealing the involvement of Nm23-H2 in tumorigenesis is from examining hepatocellular carcinomas (HCCs). Analysis of clinical samples uncovers a positive correlation of Nm23-H2 expression and primary tumor incidence in HCCs from different aspects (Table 1). With the ability to function as a NDPK and a positive association with higher tumor telomerase activity, Nm23-H2 is apparently linked to cell proliferation, tumorous

cell immortal state, and abnormal cell apoptosis in the tumorigenesis of HCCs (Iizuka et al. 2003). Using DNA microarray to compare HCCs and non-tumorous livers associated with genotype-C HBV infection, Iizuka and colleagues pinpointed Nm23-H2 (but not Nm23-H1) as one of the top 35 upregulated genes in primary HCCs (Iizuka et al. 2006). Besides Nm23-H2, the same study also found that expressions of many c-Myc-regulated genes were altered in HBV-associated HCCs. Several genes such as MCM7, PARP1, YWHAH, HSPB1, and MSH2 were upregulated, while others like MT1A and MT3 (belonging to the metallothionein

Table 1 Overview of Nm23-H2 expression level in human carcinoma cohort studies

Tumor type	Characteristics ^a	Association	Methods	References
Breast	Patients with invasive breast cancer, <i>N</i> =81	Nm23 negatively associates with survival	IHC	Cubukcu et al. 2013
CML	PBMCs from CML with Bcl-Abl (+), <i>N</i> =29	↑H2 in Bcr-Abl CML	IHC, FISH, WB	Tschiedel et al. 2012, 2008
Colorectal	TNM stage 0-IV, <i>N</i> =35	↑H2 relates to the initial tumor	IHC, IB, RT-PCR	Martinez et al. 1995
CSCC	HPV+/- cervical pap-smear, <i>N</i> =6	↑H1/2 in HPV+CSCC patients	IHC, RT-PCR	Manavi et al. 2007
	HPV+/- cervical pap-smear, <i>N</i> =3	↑H1/2 in HPV+CSCC patients	DNA microarray, IB	Hudelist et al. 2005
	Stage IB1, <i>N</i> =27	↑Nm23 with adverse prognosis	IHC, RT-PCR	Chen et al. 2001
	Dupont and Page classification, <i>N</i> =93	↓Nm23 in malignant breast lesions	IHC	Bal et al. 2008
Endometrial	<i>N</i> =71	↑H1/2 in primary tumor	IHC	Sarris and Lee 2001
	FIGO classification, <i>N</i> =40	↑H1/2 in invasive endometrial carcinoma	IHC, WB, NB	Marone et al. 1996
Gastric	Classical clinical-pathological, WHO, Lauren, Goseki, Ming classification, <i>N</i> =56	↓H1/2 expression in gastric cancer tissue than non-neoplastic mucosa	IHC	Radovic et al. 2013
	TNM, UICC-stage, <i>N</i> =116	No correlation between Nm23-H1/2 and gastric cancer stages or prognosis	IHC	Monig et al. 2007
GCT	<i>N</i> =16	↑mRNA expression in GCTSCs compared to MSCs	IHC, RT-PCR	Wuelling et al. 2004
HCC	HBV/HCV+, Edmondson-Steiner's classification, <i>N</i> =38	↑Nm23 mRNA in primary HCCs	IHC, RT-PCR	Lee et al. 2012
Lung adenocarcinomas	TNM, <i>N</i> =27	↑H1/2 in primary tumor	IB	Sato et al. 2000
MPM	Bronchial lavage, <i>N</i> =20	↑H1/2 in bronchial lavage fluid of SCLC	WB	Huwer et al. 1997
	<i>N</i> =40	↑H2 in transcript and protein levels	IHC, WB, RNA microarray	Gordon et al. 2005
NSCLC	TNM, <i>N</i> =37	↑H1/2 transcript levels in tumors	RT-PCR	Engel et al. 1993
STS	<i>N</i> =46	↑H2 in high-grade tumor	IHC	D'Souza et al. 2003
	HBV+, <i>N</i> =15	↑H2 in HCCs compared to normal liver	DNA microassay, RT-PCR	Iizuka et al. 2006

FISH fluorescence in situ hybridization, *IB* immunoblotting, *IHC* immunohistochemistry, *NB* northern blot, *WB* western blot, *AML* acute myelogenous leukemia, *CML* chronic myeloid leukemia, *CSCC* cervical squamous cell carcinoma, *GCT* giant cell tumor, *HCC* hepatocellular carcinoma, *NSCLC* non-small cell lung cancer, *MPM* malignant pleural mesothelioma, *PBMCs* peripheral blood mononuclear cells, *SCLC* squamous cell lung cancer, *STS* soft tissue sarcoma

The comparison is between primary tumor/cancer patients and normal non-tumorous tissue/healthy control

^a Characteristics of the sample such as specificities, classifications, and grades. *N* numbers of tumor samples analyzed

family) were downregulated. These observations are in keeping with the ability of Nm23-H2 to transactivate the c-Myc proto-oncogene (Postel et al. 1993). As MCM7, MSH2, and PARP1 are involved in DNA repair/maintenance and tumor progression, while metallothionein proteins are involved in the epigenetic alteration in cancer, it is plausible that the genomic instability caused by the upregulation of Nm23-H2 and c-Myc may promote tumorigenesis (Fernandez et al. 2003). Upregulation of Nm23-H2 mRNA level was similarly detected by the Bead Chip DNA microarray method in HCC samples, although the Nm23-H1 transcript level was also elevated (Lee et al. 2012). Detailed comparison between normal liver, cirrhotic liver, and graded HCC tissues indicated a positive relationship of Nm23-H2 expression and the malignancy of HCCs. Nm23-H2 protein expression is overexpressed in 75 % of the HCC tissues as well as in hepatoma cell lines (Lee et al. 2012). These observations clearly suggest a role of Nm23-H2 in the tumorigenesis of HCC associated with the major pathogen HBV, and its expression level appears to correlate positively with the pathological grading of HCC.

In cervical squamous cell carcinoma (CSCC), a similar trend of Nm23-H2 and the malignant tumor pathology has been found with DNA microarray, RT-PCR, and immunostaining methods (Table 1; Manavi et al. 2007; Hudelist et al. 2005). When cervical squamous cells from women with high-risk human papillomavirus (HPV) positive cervical carcinoma and normal ectocervical cells from HPV-negative women were analyzed by cDNA microarray, a significant increase in Nm23-H1/2 along with several other genes such as EGFR, ERBB2, K-Ras, BCL2-like 2, cyclin D1, and N-Myc (located in the nucleus) were detected in CSCC. Such correlations serve as indirect evidence of Nm23-H2 playing a role in ovarian tumorigenesis. Nevertheless, it should be noted that some of the reports were based on a small sample size (e.g., $N=6$) or they lacked supporting immunohistological data. Besides, contradictory evidence remains a big issue especially between early reports concerning the role of Nm23 genes in tumorigenesis and the prognosis of cervical cancer. It has been suggested that low Nm23 gene expression levels are associated with negative prognostic markers such as lymph node involvement or myometrial invasion (Mandai et al. 1995; Marone et al. 1996). In another study of stage IB1 cervical cancer, the survival rate of patients decreased significantly when Nm23 overexpression was observed, indicating that the expression of Nm23 might be one of the unfavorable indicator in cervical cancer prognosis (Chen et al. 2001).

There is also evidence linking the upregulation of Nm23-H2 to the initial stage of colorectal cancer (Table 1). By means of immunoblotting, immunohistochemistry, and RT-PCR, it has been shown that both the mRNA and protein levels of Nm23-H1/2 are elevated in colorectal tumors as compared

with adjacent mucosa specimen (Martinez et al. 1995; Sarris and Lee 2001). Unlike Nm23-H1, high levels of Nm23-H2 expression are sustained across early to late stages of colorectal cancer (Martinez et al. 1995). Although the significance of the differential regulation of Nm23-H1 and Nm23-H2 expression in stage III–IV tumors is unclear, elevated Nm23 expression appears to be important in early colorectal carcinoma. In contrast, the expression level of Nm23-H2 does not appear to be correlated with other cancer types such as breast, gastric, lung, pleural mesothelioma, sarcoma, and giant cell tumor (Table 1).

Contrary to the vague oncogenic role of Nm23-H2 in CSCC, considerable evidence points to a positive correlation of Nm23-H2 with hematopoietic carcinomas, especially for chronic myelogenous leukemia (CML) and acute myelogenous leukemia (AML) (Table 1). The protein expression level of Nm23-H2 is increased in mononuclear cells of peripheral blood from pre-transplant Philadelphia chromosome-positive CML patients compared with the control HLA-identical donor, with an interesting co-localization of Nm23-H2 and β -actin in the cytoplasm (Tschiedel et al. 2008). Furthermore, Nm23-H2 overexpression correlates closely with the activity of the oncogenic Bcr-Abl fusion protein, a tyrosine kinase which acts as a GTPase-activating protein for Rac1 and Cdc42, independent of HLA type (Tschiedel et al. 2012). The expression of Nm23-H2 is high in peripheral blood cells of tyrosine kinase inhibitor imatinib-resistant patients with CML and low in cells of responding patients. Nm23-H2 overexpression is therefore regarded as a common characteristic of CMLs and is potentially relevant to both the molecular basis and immune therapy of the disease. Moreover, the overexpression of either of the closely related Nm23-H1/2 genes in AML has been reported to be associated with a lower survival rate (Okabe-Kado et al. 1998, 2002). Early studies have investigated the relative levels of Nm23-H1/2 and c-Myc transcripts in bone marrow and peripheral blood samples from 110 patients with AML using the RT-PCR (Okabe-Kado et al. 1998). The expression levels of Nm23-H1/2 in the AML samples were significantly higher than in normal blood cells, and an elevated level of Nm23-H2 expression was correlated with adverse prognosis for AML patients in a subtype-specific pattern (Okabe-Kado et al. 1998). A subsequent study further characterized cell surface Nm23-H1/2 proteins as a marker of leukemia cells derived from various cellular lineages and differentiation stages (Okabe-Kado et al. 2002).

Intriguingly, extracellular Nm23-H1/2 proteins have been detected in conditioned medium of cell lines and body fluids from cancer patients with malignant hematologic malignancies, breast, colon, pancreas, or lung tumors (Anzinger et al. 2001; Rumjahn et al. 2007; Niitsu et al. 2000, 2001; Okabe-Kado and Kasukabe 2003). It has been reported that one of the

differentiation inhibitory factors present in the conditioned medium and the membrane fraction of a non-differentiating mouse myeloid leukemia M1 cell line is Nm23-M2 (Okabe-Kado et al. 1992). It was subsequently demonstrated that exogenously added recombinant proteins of different Nm23 isoforms (Nm23-H1/2 and Nm23-M1/2) are able to inhibit the induction of erythroid differentiation in several human leukemia cell lines (Okabe-Kado et al. 1995a). These studies suggest that Nm23-H1/2 can inhibit hematopoietic differentiation. This inhibitory activity of Nm23-H1/2, however, is apparently independent of the NDPK activity (Okabe-Kado et al. 1995b). In line with this notion, endogenous gene expressions of Nm23-H1 and Nm23-H2 are downregulated during *in vitro* differentiation of AML cells (Yamashiro et al. 1994 Caligo et al. 1996), and Nm23-H1 and Nm23-H2 are highly expressed in normal hematopoietic progenitors but are downregulated during normal hematopoietic maturation (Willems et al. 1998). Besides hematopoietic cells, Nm23 protein can be detected in the conditioned medium of other tumor cell lines. As Nm23-H2 is secreted as a phosphoprotein, it may perform transphosphorylation in the absence of an extracellular phosphoryl donor to generate NTP which can then activate cell surface receptors (e.g., purinergic P2Y receptors) to regulate cellular functions. In this regard, extracellular Nm23 protein secreted by human breast cancer cells has been shown to promote *in vitro* angiogenesis via P2Y receptor activation (Rumjahn et al. 2007). Despite these efforts, there is little experimental data supporting a possible mechanism which can correlate the upregulation of Nm23-H2 with leukemia, or the dynamic changes in mRNA/protein levels of Nm23-H2 with the different phases of CML/AML.

In vivo/vitro study of Nm23-H2 in tumorigenesis-associated processes

In the absence of a clearly defined mechanism, it is hard to assess the contribution of Nm23-H2 in the tumorigenesis of human malignancies, although there are multiple attempts to clarify this issue using human cancer cell lines and xenograft nude mice models (Table 2). In cell lines such as HLK3 hepatocytes and NIH3T3 fibroblasts, ectopic expression of Nm23-H2 leads to a transformed morphology, enhanced focus formation, and anchorage-independent growth (Lee et al. 2012). Athymic mice injected with NIH3T3 fibroblasts and HLK3 hepatocytes stably expressing Nm23-H2 produced tumors with elevated c-Myc and cyclin D1 expression. In line with these observations, lentiviral delivery of shRNA against Nm23-H2 inhibited tumor growth of xenografts produced from HLK3 hepatocytes stably expressing Nm23-H2 (Lee et al. 2012). Both c-Myc and cyclin D1 have been suggested to be closely related to the tumorigenesis of HCC. In particular, c-Myc appears to be a frequently activated oncogene in

HCC (Murakami et al. 1993), while protein microarray analysis has revealed an upregulation of cyclin D1 in HCC compared with normal liver (Tannapfel et al. 2003). Collectively, these results tend to indicate a pro-oncogenic role of Nm23-H2 in hepatocarcinogenesis which appears to involve c-Myc expression. As discussed below, Nm23-H2 can indeed act as a transcriptional activator of oncogenic c-Myc.

Although Nm23-H2 is generally believed not to exert any tumorigenic effect in human melanoma, there is evidence to suggest its involvement in early or pro-metastasis cascade. In mouse models of UV radiation or phorbol ester-induced melanomas, expression of murine Nm23-M2 is upregulated relative to adjacent normal skin (Wei et al. 2004). Functional studies further suggest that the Nm23-M2 overexpression enhances neoplastic transformation in mouse epidermal cells (Wei et al. 2004). In another study focusing on human melanoma metastasis, the growth rate of tumors derived from transfectants expressing a functionally defective Nm23-H2 mutant (NDPK B/T) was roughly twice that of tumors established from Nm23-H1 expressing lines (Hamby et al. 2000). Likewise, the tumorigenic potential of Nm23-H2 has been demonstrated in murine models and cell lines for mammary and oral squamous cell carcinoma (Table 2).

Contrastingly, Nm23-H2 appears to act as a negative regulator in ovarian cancer. Nm23-H2 was identified by cDNA microarray as one of several genes that were upregulated by progesterone and exhibited anti-ovarian cancer action (Table 2; Syed et al. 2005). As compared to immortalized non-tumorigenic human ovarian surface epithelial (HOSE) cell lines, Nm23-H2 is downregulated in ovarian cancer (OVCA) cell lines but its level can be restored upon progesterone treatment for 5 days (Syed et al. 2005). Moreover, ectopic expression of Nm23-H2 caused growth inhibition and suppression of neoplastic transformation in OVCA cell cultures, but not in HOSE cell cultures. These observations are in agreement with earlier reports that showed reduced Nm23-H2 expression in ovarian cancer tissues from patients with metastatic lymph node (Mandai et al. 1995), as well as an association of higher levels of Nm23-H2 expression with better prognosis in ovarian cancer patients (Tas et al. 2002). However, contradictory results from breast/mammary, colorectal, and oral squamous carcinoma imply complicated function of Nm23-H2 in different cellular backgrounds (Table 2). Additional studies are needed to further illustrate the importance of Nm23-H2 in tumorigenesis-related biological processes.

Nm23-H2 interaction partners in various biological processes

Although several interaction partners of Nm23-H1 (e.g., plakoglobin, KSR1, MUC1*, HPV 16E7, see Table 3) appear to have similar interplay with Nm23-H2, many components

Table 2 Trends of the tumorigenic effect by Nm23-H2 expression in tumor cell lines

Cell types	Cell lines	Association	Methods	References
In vivo^a				
Breast/mammary	MTLn3, MTC	MTLn3-H1/2 were tumorigenic in syngeneic rats	WB, xenograft assay	Fukuda et al. 1996
Liver	NIH3T3, HEK293T, Hep3B, PLC/PRF/5, HepG2, SK-HEP-1, Huh7, Rat-1	H2 overexpression results in tumor in mice	Nude mice xenograft, IHC, soft agar	Lee et al. 2012
Melanoma	Murine B16-FE7	No change in primary tumor size	Xenograft, proliferation assay	Baba et al. 1995
	Line IV C1 1	Nm23-H2/T ^b significantly promotes primary tumor growth	Xenograft, WB, RT-PCR	Hamby et al. 2000
Skin	JB6 Cl 41-5a, JB6 Cl 30-7b, Rat-1, HaCaT, A431, Tg-AC43, FVB/N217, NIH/3 T3	UV/TPA-induced Nm23-H2 leads to epidermal neoplastic transformation	Animal model, transformation assay, RT-PCR	Wei et al. 2004
In vitro				
Colorectal	Rat colon 26	No change in proliferation of H2-stable line	Proliferation assay	Tagashira et al. 1998
Leukemia	AML-M6	Expression of H2 in AML cell lines	FACS analysis, Complement-dependent cytotoxicity	Okabe-Kado et al. 2002
Oral squamous	HSC-2, HSC-4, LMF4, LMF5	H2-stable line increases cell growth	WB, IHC	Miyazaki et al. 1999
Ovarian	OVCA, HOSE	Ectopic expression of H2 inhibit OVCA growth	Proliferation assay, soft agar	Syed et al. 2005
	SKOV3, SKOV3.ip1	More aggressive line has lower Nm23-H2 gene expression	DNA microarray, Matrigel, WB	Bai et al. 2006
	CAL 27	H2 might have an important role in oral squamous cell carcinoma	DNA microarray	Herak Bosnar et al. 2008

FACS fluorescence-activated cell sorting, *A431* human epidermoid carcinoma, *AML-M6* acute myelogenous leukemia-M6 subtype, *B16-FE7* highly metastatic mouse melanoma, *CAL 27* oral squamous cell carcinoma of the tongue, *FVB/N217* FVB/N carcinoma, *HEK293T* human embryonic kidney 293, *HaCaT* human keratinocyte, *HepG2* human hepatocellular carcinoma, *Hep3B* human hepatocellular carcinoma, *HOSE* human ovarian surface epithelial, *HSC-2*, *HSC-4*, *LMF4*, *LMF5* human oral squamous, *Huh7* human hepatocarcinoma, *JB6 Cl 41-5a*, *JB6 Cl 30-7b*, TPA-susceptible/resistant mouse epidermal cells, *Line IV C1 1* human melanoma, *MSCs* Mesenchymal stem cells, *MTC*, *MTLn3* rat mammary-adenocarcinoma cell lines of poor and high metastatic potential, respectively, *NIH3T3* mouse embryonic fibroblast, *OVCA* ovarian cancer, *PLC/PRF/5* *Homo sapiens* liver hepatoma, *Rat-1* hepatoma/glioma, *SK-HEP-1* *Homo sapiens* liver/ascites adenocarcinoma, *SKOV3* human ovarian cancer, *Tg-AC43* TPA-induced Tg.AC squamous cell carcinoma

^aXenograft in mice with cancer cell lines

^bNDPK inactive form of Nm23-H2

that are known to interact with Nm23-H1 either fail to recognize Nm23-H2 or such interaction have yet to be explored (Table 3). For example, the guanine nucleotide exchange factor (GEF) Dbl-1 can interact with Nm23-H1 and as a consequence inhibits the small GTPase Cdc42-mediated cytoskeleton reorganization, while the same situation does not apply to Nm23-H2 (Murakami et al. 1993). On the other hand, only Nm23-H2 appears to bind with MDM2 (an E3 ubiquitin-protein ligase) specifically and this binding inhibits the Nm23-H2 anti-motility effect in a p53-independent manner (Polanski et al. 2011).

To appreciate the diversity of interacting partners unique to Nm23-H2 as well as those shared with Nm23-H1, a list of known protein partners for Nm23-H2 is shown in Table 3.

Several of these interacting partners are indeed involved in the anti-metastasis function (e.g., Lbc) while others appear to regulate different physiological/pathological processes (e.g., Kca3.1 and PRE). However, accumulating evidence has linked a number of proto-oncogenes, oncogenes, and tumor suppressor genes with Nm23-H2. A prime example is MDM2, a proto-oncogene known for its ability to downregulate p53 tumor suppressor. H1299 cells expressing high levels of MDM2 are non-responsive to Nm23-H2-mediated suppression of motility, suggesting a role of MDM2 to override the motility suppressing activity of Nm23-H2 (Polanski et al. 2011). MDM2 displays oncogenic effect and serves as an adverse prognosis indicator in a range of common human

Table 3 Nm23-H2 interaction partners and phenotypes in physiological/pathological processes

Partners	Assays	Cell lines	Functions	References
DNA-protein				
TRF1	Y2H, EMSA, Co-IP	HeLa	Telomerase	Nosaka et al. 1998
TRF2	Ch-IP, Co-IP	A549, HT-1080	Telomerase	Kar et al. 2012
Protein-protein				
Diva	Y2H, IF, IP	SKOV3, 293T, HeLa, PC12	Apoptosis; differentiation	Kang et al. 2007; Lim et al. 2012
ER β	Y2H, pull-down	HeLa, MCF-7	Transcription; cell migration	Rayner et al. 2007, 2008
G β γ /caveolae	IF, IB, Co-IP	TE4/6 Zebrafish <i>Danio rerio</i>	Signal transduction	Wieland 2007, Hippe et al. 2009, 2011
HPV 16E7 ^a	Y2H, Co-IP	PC12	Transcription; protein degradation	Mileo et al. 2006
Icap-1 α	Co-IP	ES, iPS	Cell migration	Fournier et al. 2002, Miyamoto et al. 2009
KCa3.1	Y2H, WB, flow cytometry	CD4 T cells	Channel activation	Srivastava et al. 2006, Ohya et al. 2014
KSR1 ^a	Co-IP	HEK293	Signaling inhibition	Tso et al. 2013 (Fig. S2)
Lbc	Co-IP	COS7	Cell skeleton	Iwashita et al. 2004
MDM2	Y2H, Co-IP	H1299, MCF7, HEK293, UOK117	Metastasis	Polanski et al. 2011
MUC1 ^{*a}	SPR	ES, iPS	Cell proliferation; differentiation	Mahanta et al. 2013, Smagghe et al. 2013
PIWIL2	Co-IP, IF	HeLa, HepG2, HEK293	Gene expression	Yao et al. 2014
Plakoglobina	IF, Co-IP	SK-OV3, HEK293T, HeLa	Metastasis	Aktary et al. 2010
ROR α^a	Y2H, pull-down	Y190	Unknown	Paravicini et al. 1996
RZR β	Y2H, pull-down	Y190	Unknown	Paravicini et al. 1996
TP β	Y2H, Co-IP, IF	HEK293	Receptor regulation	Rochdi et al. 2004

Co-IP co-immunoprecipitation, *Ch-IP* Chromatin immunoprecipitation, *EMSA* electrophoretic mobility shift assay, *IF* immunofluorescence, *SPR* surface plasmon resonance, *Y2H* yeast two-hybrid, *A549* human lung adenocarcinoma epithelial cell line, *COS7* monkey kidney fibroblast-like cell line, *ES* Embryonic stem cells, *H1299* human non-small cell lung carcinoma cell line, *HeLa* human cervical carcinoma cell line, *HEK293* human embryonic kidney 293 cell line, *HT-1080* human fibrosarcoma cell line, *iPS* Induced-pluripotent stem cells, *MCF-7* human breast adenocarcinoma cell line, *PC12* rat adrenal medulla cell line, *SK-OV3* human ovarian carcinoma cell line, *UOK 117* renal cell carcinoma cell line, *Y190* yeast strain

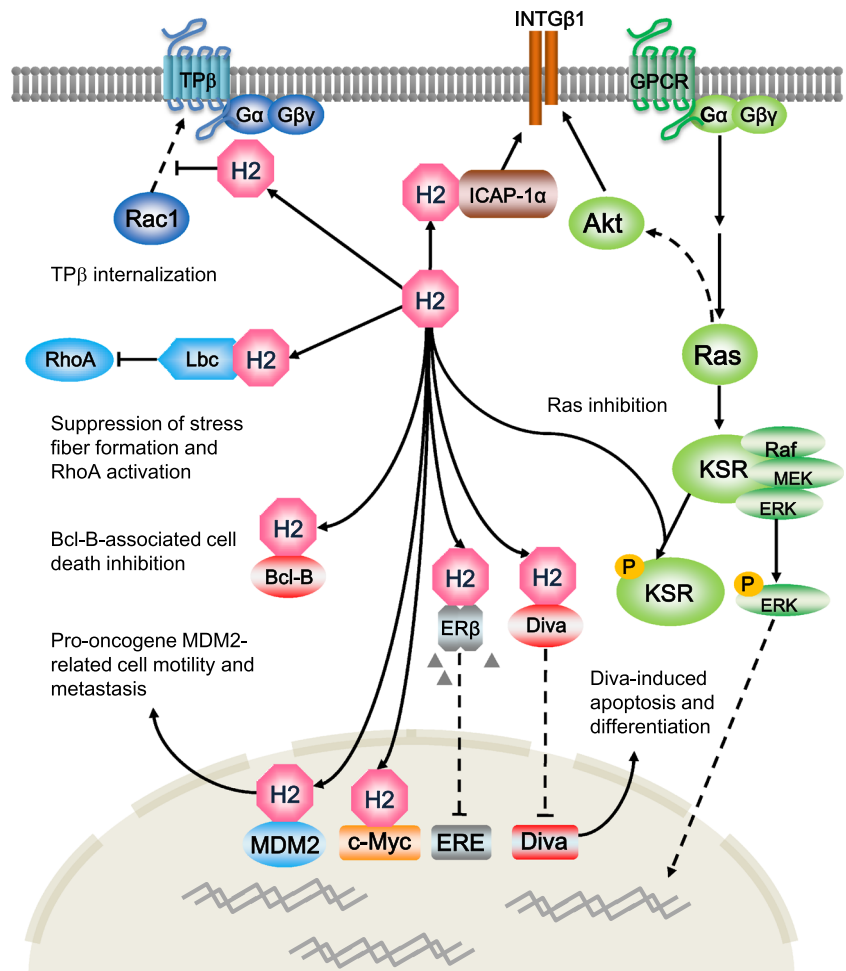
^a Also acts as an interacting partner of Nm23-H1

cancers including prostate, bladder, esophageal and renal cancers (Haitel et al. 2000). In addition, various reports (see Table 3) have shown that the RhoA family member associated regulatory factors such as Lbc and ICAP-1 α (integrin cytoplasmic domain-associated protein 1 α) are capable of interacting with Nm23-H2 (Fig. 2). Nm23-H2 can specifically bind to Lbc at the amino-terminal region and this interaction has been shown to occur independently of kinase activity. Moreover, the level of GTP-bound Rho could be downregulated by the expression of Nm23-H2 and subsequently decrease stress fiber formation stimulated by Lbc (Iwashita et al. 2004). Yeast two-hybrid screen has identified Nm23-H2 as a partner of ICAP-1 α , and their interaction has been confirmed by using purified recombinant ICAP-1 α and Nm23-H2 in pull-down assays. Confocal fluorescence microscopy further demonstrated co-localization of ICAP-1 α and Nm23-H2 in the lamellipodia during early stages of CHO cell spreading

(Fournier et al. 2002). A tertiary complex formed by Nm23-H2, Lbc, and ICAP-1 α is thought to be critical for cell migration and morphological changes stimulated by integrin (Miyamoto et al. 2009). Lbc has been identified as an oncogene from human leukemic cells and ICAP-1 α can stimulate proliferation of epithelial cells (Toksoz and Williams 1994; Fournier et al. 2005). Such interactions are unique to Nm23-H2 but it remains to be determined if recognition by Nm23-H2 results in functional enhancement or impairment of these proliferation-related proteins.

Diva (death inducer binding to vBcl-2 and Apaf-1)/Bcl-B is a Bcl-2 apoptosis family member with contradictory roles in the regulation of cell cycle and apoptosis. There is evidence to suggest that Nm23-H2, but not H1, binds to Diva directly and the two proteins are colocalized in the cytoplasm of SK-OV3 cells (Kang et al. 2007). Moreover, coexpression of Nm23-H2 tends to lower the expression of Diva while silencing Nm23-

Fig. 2 Schematic diagram to illustrate Nm23-H2 and its interacting partners in a variety of cellular events. Nm23-H2 (pink) is able to directly bind to or indirectly affect a wide range of proteins or genes highlighted with different colors, which are mainly involved in signal cascading (green), adhesion (brown), motility (cyan), gene transcription (gray), endocytosis (deep blue), as well as the cell cycle (red). Nm23-H2 may play an indirect role in activating the transcription of c-Myc gene (gold). Dashed lines indicate indirect crosstalk of inhibition or activation. See text for detailed information on different Nm23-H2 interacting partners



H2 effectively rescues Diva expression. This suggests that Nm23-H2 may modulate the Diva-associated apoptosis via a complex mechanism involving protein-protein interaction (Fig. 2). In addition, Nm23-H2 can downregulate the expression of Bcl2L10, a human ortholog of Diva (Xu et al. 2011). Another study has shown that the Diva/Nm23-H2 complex arrests cell cycle progression in PC-12 cells, whereas the overexpression of Diva had an opposite effect (Lim et al. 2012). Further investigations are needed to test if Nm23-H2 can indeed facilitate tumorigenesis by inhibiting apoptosis.

It is well established that estrogen can stimulate the growth of some tumors including endometrial cancer. Nm23-H2 was identified as an ER β -associated protein in a yeast two-hybrid screen in the context of vascular atherosclerosis disease (Rayner et al. 2007, 2008). Some evidence indicates close relationship between ER β and Nm23-H2. Firstly, treatment with ER β -selective agonist increases expression of Nm23-H2 in human coronary smooth muscle cells, while this effect is not seen with ER α -selective agonist (Rayner et al. 2007). Secondly, Nm23-H2 and ER β consistently co-localize in a variety of human tissues including breast tissue, whereas ER α and Nm23-H2 do not seem to co-localize. Estrogen also prompted

nuclear localization of Nm23-H2 protein in smooth muscle cells (Rayner et al. 2008). More importantly, Nm23-H2 overexpression synergizes with estrogen treatment in suppressing MCF-7 cell migration, which suggests a regulatory role of Nm23-H2 and estrogen-associated response in cell migration. ER β is downregulated and is associated with good clinical outcome in ovarian but not in endometrial cancer (Haring et al. 2012). It is worthy to further investigate the relationship of ER β and Nm23-H2 in estrogen-related cancers such as ovarian and breast carcinomas.

Ras/ERKs signaling play a critical role in various biological processes including cell proliferation, differentiation, motility, and survivals (Shaul and Seger 2007). Many abnormal cellular compositions and microenvironment can lead to tumorigenesis by altering Ras signaling. Like Nm23-H1, Nm23-H2 is evidently linked to the suppression of Ras-related pathways (Lee et al. 2009; Masoudi et al. 2013). In both NIH3T3 and HEK293 cells, knockdown of Nm23-H2 significantly enhances the activity of ERK whereas overexpression of Nm23-H2 reverses this trend (Lee et al. 2009). These observations imply that Nm23-H2 may serve as a negative regulator of the Ras/MEK/ERK pathway which is

typically stimulated by growth factors and mitogens. The mechanism by which Nm23-H2 (as well as Nm23-H1) suppresses Ras signaling is via the phosphorylation and inactivation of a scaffolding protein known as KSR1 (kinase suppressor of Ras 1). KSR1 has been identified as a common binding partner of Nm23-H1 and Nm23-H2 (Hartsough et al. 2002; Tso et al. 2013) is essential for the assembly of the Raf/MEK/ERK kinase cascade upon Ras activation. Overexpression of Nm23-H1 results in higher histidine protein kinase activity, which phosphorylates KSR and eventually leads to its degradation, thus suppressing its ability to facilitate Ras activation (Hartsough et al. 2002). Given that Ras-related growth signals is a common mechanism across different cellular backgrounds, KSR/Nm23-H2 interaction is likely to exert a growth inhibitory effect in many cell types. Yet, Nm23-H2-induced growth inhibition is not generally observed. This tends to suggest that Nm23-H2 may also elicit growth promoting actions (e.g., induction of c-Myc) to counter balance the suppression of Ras signaling. Lastly, a number of studies have described the ability of Nm23-H2 to interact with G protein-related partners (Table 3). These include the formation of caveolin/G β -subunit/Nm23-H2 complex in the maintenance of intracellular membrane compartments, and a TP β (thromboxane A2 receptor β)-Nm23-H2 complex in receptor endocytosis through Rac1 signaling (Hippe et al. 2011, 2011; Rochdi et al. 2004). Apart from direct interaction, Nm23-H2 (also Nm23-H1) could be upregulated by regulator of G protein signaling 19 (RGS19) via an unknown mechanism, leading to the inhibition of Ras signaling (Tso et al. 2013). Although these interplays are not directly associated with tumorigenesis, G protein signals are known to modulate cell growth and cell cycle progression (New and Wong 2007).

Transcriptional regulation of the c-Myc oncogene

It has long been known that Nm23-H2 can activate c-Myc transcription independently of its catalytic function, and this ability is surprisingly not shared by Nm23-H1 (Postel et al. 1993; Berberich and Postel 1995). Nm23-H2 is a major regulator of c-Myc transcription since its knockdown leads to the downregulation of c-Myc (Dexheimer et al. 2009). RNA silencing experiments showed that Nm23-H2 binds to both the single-stranded guanine- and cytosine-rich strands of the c-Myc NHE III, but not to the duplex form. A nuclease hypersensitive element (NHE) located in the promoter region of the Myc gene appears to be critical for the transactivation of c-Myc by Nm23-H2, as demonstrated by a Myc-CAT reporter plasmid with this element deleted (Berberich and Postel 1995). And mutagenesis of Nm23-H2 has identified several residues on the equator of the hexameric protein for sequence-dependent DNA binding (Postel et al. 2002). Further research attempting to illustrate this transcriptional regulation on c-

Myc focused on a G-quadruplex structure (G4 motif) and concluded that the changed G4 motif by Nm23-H2 increases the transcription of c-Myc (Postel et al. 2000). However, it was then suspected that the proposed DNA cleavage activity of Nm23-H2 was related to a contaminant during protein purification (Dexheimer et al. 2009). Moreover, at least one major publication on the issue of G-quadruplex element and Nm23-H2 have been retracted, so there remains a lot of controversies and uncertainties in the regulation of c-Myc transcription by Nm23-H2 (Stegg et al. 2011). From this perspective, reports linking Nm23-H2 to c-Myc and G4 motif may need to be re-examined carefully, such as the G4-related CNBP/Nm23-H2 interaction (Chen et al. 2013) and competitive DNA binding effect of Nm23-H2/IFI16 (Egistelli et al. 2009). Considering the close relationship between c-Myc and tumorigenesis, any future study regarding the role of Nm23-H2 in tumorigenesis via c-Myc activation should be conducted more prudently.

Nm23-H2 in multi-potent stem cell differentiation and hematopoietic maturation

The concept of cancer stem cells driving tumorigenesis has received considerable support in recent years. In this regard, it is fascinating to note that Nm23-H1 has been shown to maintain the “stemness” of pluripotent stem cells (Smagghe et al. 2013). Dimeric extracellular Nm23-H1 in a fully defined xeno-free medium can apparently allow serial passaging of both human embryonic stem (ES) cells and induced-pluripotent stem (iPS) cells (Hikita et al. 2008). Gene expression profiling further indicates that stem cells cultured in the presence of Nm23-H1 can easily maintain a “naïve” state (Smagghe et al. 2013). As a putative stem cell growth factor, extracellular Nm23-H1 is presumed to act on a cell surface component. This putative receptor has been suggested to be MUC1*, a transmembrane cleavage product of Mucin 1 (MUC1) which is aberrantly expressed in around 75 % of all human solid tumors. Binding of extracellular Nm23-H1 to MUC1* stimulates the growth of human ES cells (Hikita et al. 2008). Although these studies did not address whether Nm23-H2 can similarly support the proliferation of ES cells, Nm23-H2 has been shown to bind MUC1* as efficiently as Nm23-H1 (Mahanta et al. 2008). It remains to be determined if Nm23-H2 and MUC1* can cooperate to control stem cell self-replication, promote growth, and maintain pluripotency. An indirect line of evidence supporting the involvement of Nm23-H2 in the maintenance of pluripotency has been obtained through high-content screening of novel factors that control lineage commitment in ES cells. Stauprimide, a small molecule which facilitates differentiation of mouse and human ES cells, is found to interact with Nm23-H2 and inhibits its nuclear translocation (Zhu et al. 2009). Although the

mechanism remains unclear, this represses c-Myc expression and leads to a reduction in the level of c-Myc. Since c-Myc is a key factor in self-renewal of ES cells, its downregulation enhances the efficiency of directed differentiation of ES cells. Collectively, these studies point to an important role of Nm23-H2 in the maintenance of the undifferentiated state. It is noteworthy that Nm23-H2 is highly expressed in undifferentiated ES cells, but its expression decreases upon differentiation.

As accumulating evidence suggests that malignant transformation of hematopoietic stem cells is the main cause in leukemogenesis, comparative studies on Nm23 protein expression in hematopoietic cells may provide insightful clues on the involvement of Nm23-H2 in leukemogenesis. By flow cytometric analysis and enzymatic assays, an early study has indeed noted changes in the expression of Nm23 isoforms during bone marrow maturation (Willems et al. 1998). Nm23-H2 is downregulated during maturation of CD34⁺ bone marrow cells and recombinant Nm23-H2 is able to inhibit the differentiation of human leukemia cell lines as well as normal hematopoietic cells (Okabe-Kado et al. 1995a; Willems et al. 2002). However, it should be noted that other isoforms of Nm23 can also inhibit differentiation in a human erythroid leukemia model (Yokoyama et al. 1996). Evidence from another study focusing on the translational control of Nm23-M2 showed that the expression of endogenous Nm23-M2 declined via a panel of altered cytokines during erythroid progenitor differentiation (Joosten et al. 2004).

By means of serum-free colony-forming unit assays, it has been shown that Nm23-H1, Nm23-H2, and Nm23-H3 can alter the differentiation of more mature bone marrow progenitor CD34⁺CD38⁻ cell lineage than the immature CD34⁺CD38⁻ population (Willems et al. 2002). This effect is independent of the NDPK activity since the enzymatically inactive H118N mutant of Nm23-H1 produces a similar result. A recent study using Nm23-H1^{-/-}/Nm23-H2^{-/-} double knockout mice has further documented the abnormal hematopoiesis and defect in erythroid development (Postel et al. 2009). These mice show a series of hematological phenotypes that are indicative of an abnormal immature state in erythropoiesis. The significance of these findings lies in the fact that the level of Nm23-H2 is upregulated in human hematological malignant leukemic cells and in abnormal erythropoiesis. The correlation of high levels of Nm23-H1/2 in the plasma of leukemia patients has prompted the pursuit of Nm23-H1/2 as a prognostic factor in hematological malignancies (Okabe-Kado et al. 1998; Niitsu et al. 2001).

Nm23-H2 may also be involved in neuronal differentiation. Overexpression of Nm23-M1 protein induces neurite outgrowth and expression of neurofilament, whereas its downregulation inhibits neuronal differentiation (Gervasi et al. 1996). A recent study proposes that the Diva/Bcl-B complex competes against β -tubulin for Nm23-H2 and prevents the

latter from translocating to the nucleus (Table 3; Lim et al. 2012). Overexpression of Nm23-H2 promotes PC-12 neuronal differentiation by increasing neurite outgrowth and arresting cell cycle progression. During differentiation, Diva/Bcl-B expression and Nm23-H2 level display an inverse relationship with the former decreasing while the latter increases (Lim et al. 2012). Interestingly, high Nm23-H1 levels have been reported to correlate with disease aggressiveness and survival reduction for patients with neuroblastoma (Hailat et al. 1991) and a mutation at a conserved serine (S120G), which prevents histidine-dependent protein phosphotransfer reactions, is found in advanced stages of the disease (Chang et al. 1994). As a result, Nm23-H1 has become one of three genes adopted for risk stratification of patients with neuroblastoma (Garcia et al. 2012). Because early studies were primarily focused on Nm23-H1, it remains unclear if Nm23-H2 is similarly associated with neuroblastomas. The observation that N-Myc is upregulated in advanced neuroblastoma (Hailat et al. 1991) tends to suggest the involvement of Nm23-H2 because Nm23-H1 does not induce transcription of the Myc gene. However, it should also be noted that Nm23-H1 and Nm23-H2 represent strongly upregulated downstream targets of N-Myc, and the combined elevation of N-Myc and Nm23-H1/2 appears to inhibit neuronal differentiation by suppressing the function of Cdc42 (Valentijn et al. 2005).

Modification of telomerase activity by Nm23-H2

One mechanism by which cancer cells acquire immortalization is through the activation of telomerase to maintain the telomere length (van Steensel and de Lange 1997). Cell senescence can be effectively circumvented by preventing the loss of genetic information upon shortening of the telomeres following repeated DNA replication. The regulation of telomerase activity is highly complex and involves multiple components. Telomere repeat binding factor 1 (TRF1) is a double-stranded telomeric binding protein which limits telomere elongation by telomerase in immortalized human cell lines. As a suppressor of telomere elongation, TRF1 is involved in a negative feedback mechanism that stabilizes telomere length in several kinds of human cancers (Donate and Blasco 2011). In vitro binding assays using different recombinant Nm23 isoforms have shown that TRF1 predominantly binds to Nm23-H2 rather than Nm23-H1 (Nosaka et al. 1998). Interestingly, the in vitro affinity of telomerase for its substrate is increased in the presence of excess Nm23-H2 (Nosaka et al. 1998). Although it is entirely plausible that the suppressive function of TRF1 on telomerase activity is impaired upon binding to Nm23-H2, there is no follow-up study. Nevertheless, a recent study using Ch-IP-seq in human lung adenocarcinoma and fibrosarcoma cells confirms that Nm23-H2 can indeed bind to the telomeres in vivo (Kar et al. 2012).

The presence of Nm23-H2 at telomeric ends has been validated and additional evidence indicates the association of Nm23-H2 and TRF2 in the nucleus. Remarkably, Nm23-H2 inhibits telomerase independently of its nuclease activity in vitro and in vivo, and sustained Nm23-H2 expression leads to reduced telomere length in aggressive human cancer cells (Kar et al. 2012). Despite the contradicting evidence of Nm23-H2 enhancing the formation of telomerase/substrate complex (Nosaka et al. 1998) while reducing telomerase activity (Kar et al. 2012), the unique ability of Nm23-H2 to associate with TRF1/2 suggests a possible link to tumorigenesis via modulation of telomerase. Given that a telomerase activator, cycloastragenol, efficiently stimulates ERK phosphorylation (Yung et al. 2012) and that ERK-responsive transcription factors such as Ets2 and ER81 can upregulate telomerase expression and activity (Maida et al. 2002; Goueli and Janknecht 2004), the ability of Nm23-H2 to regulate tumorigenesis via modulation of telomerase activity will be negatively affected by its effect on Ras signaling. Besides, the G4 motif mentioned above has been found in the telomere end of human genome (Neidle 2010), which makes the unclarified relationship between Nm23-H2, G4 motif, and telomere more confounding and fascinating. Clearly, much more effort is needed to decipher the true significance of the binding of Nm23-H2 to the telomeres.

Conclusion

Despite extensive efforts to unravel the biological functions of Nm23 proteins, the ability of Nm23-H2 to regulate tumorigenesis remains somewhat controversial. Nevertheless, considerable experimental and clinical evidence are available to support the notion that Nm23-H2 is more than just a metastasis suppressor; it may be capable of promoting and modulating tumorigenesis under different cellular environments. The major difficulty in discerning its actions on tumorigenesis is the multifunctional nature of Nm23-H2 as well as its ability to form heterohexamers with Nm23-H1. Nm23-H2 can regulate numerous signaling networks and many of which have established links to tumorigenesis. Transcriptional activation of c-Myc is obviously pro-oncogenic and appears to underlie the association of certain cancer types with high levels of Nm23-H2, while suppression of Ras signaling by Nm23-H2 is expected to inhibit tumorigenesis. Nm23-H2 can potentially modulate cell growth or differentiation by interacting with a plethora of signaling molecules that range from cell surface and intracellular receptors (e.g., MUC1* and ER β) to cytosolic and nuclear proteins (e.g., Lbc and TRF1/2). New strategies are required to tease out the different functions of Nm23-H2 in tumorigenesis, and the use of a small number of model systems may facilitate data interpretation by limiting or maintaining the number of partners available to Nm23-H2.

Acknowledgments Supported by the National Key Basic Research Program of China (2013CB530900), Hong Kong RGC (663110 and 663412), UGC (T13-607/12R), and the Hong Kong Jockey Club.

Conflict of interest statement The authors declare no conflict of interest.

References

- Aktary Z, Chapman K, Lam L et al (2010) Plakoglobin interacts with and increases the protein levels of metastasis suppressor Nm23-H2 and regulates the expression of Nm23-H1. *Oncogene* 29:2118–2129
- Anzinger J, Malmquist NA, Gould J, Buxton IL (2001) Secretion of a nucleoside diphosphate kinase (Nm23-H2) by cells from human breast, colon, pancreas and lung tumors. *Proc West Pharmacol Soc* 44:61–63
- Arnaud-Dabernat S, Bourbon PM, Dierich A, Le Meur M, Daniel JY (2003) Knockout mice as model systems for studying nm23/NDP kinase gene functions. Application to the nm23-M1 gene. *J Bioenerg Biomembr* 35:19–30
- Baba H, Urano T, Okada K et al (1995) Two isotypes of murine nm23/nucleoside diphosphate kinase, nm23-M1 and nm23-M2, are involved in metastatic suppression of a murine melanoma line. *Cancer Res* 55:1977–1981
- Bai F, Feng J, Cheng Y, Shi J, Yang R, Cui H (2006) Analysis of gene expression patterns of ovarian cancer cell lines with different metastatic potentials. *Int J GynecolCancer* 16:202–209
- Bal A, Joshi K, Logasundaram R, Radotra BD, Singh R (2008) Expression of nm23 in the spectrum of pre-invasive, invasive and metastatic breast lesions. *Diagn Pathol* 3:23
- Berberich SJ, Postel EH (1995) PuF/NM23-H2/NDPK-B transactivates a human c-myc promoter-CAT gene via a functional nuclease hypersensitive element. *Oncogene* 10:2343–2347
- Boissan M, Dabernat S, Peuchant E, Schlattner U, Lascu I, Lacombe ML (2009) The mammalian Nm23/NDPK family: from metastasis control to cilia movement. *Mol Cell Biochem* 329:51–62
- Caligo MA, Cipollini G, Petrinit M, Valentini P, Bevilacqua G (1996) Down regulation of NM23.H1, NM23.H2 and c-myc genes during differentiation induced by 1,25 dihydroxyvitamin D3. *Leuk Res* 20:161–167
- Chang CL, Zhu XX, Thoraval DH et al (1994) Nm23-H1 mutation in neuroblastoma. *Nature* 370:335–336
- Chen HY, Hsu CT, Lin WC, Tsai HD, Chang WC (2001) Prognostic value of nm23 expression in stage IB1 cervical carcinoma. *Jpn J Clin Oncol* 31:327–332
- Chen S, Su L, Qiu J et al (2013) Mechanistic studies for the role of cellular nucleic-acid-binding protein (CNBP) in regulation of c-myc transcription. *Biochim Biophys Acta* 1830:4769–4777
- Chen Y, Gallois-Montbrun S, Schneider B et al (2003) Nucleotide binding to nucleoside diphosphate kinases: X-ray structure of human NDPK-A in complex with ADP and comparison to protein kinases. *J Mol Biol* 332:915–926
- Cubukcu E, Kanat O, Fatih Olmez O et al (2013) Prognostic significance of estrogen receptor, progesterone receptor, HER2/neu, Ki-67, and nm23 expression in patients with invasive breast cancer. *J BUON* 18:359–365
- Dexheimer TS, Carey SS, Song ZH et al (2009) While NM23-H2 may play an indirect role in transcriptional activation of c-myc gene expression, it does not cleave the NHE III1 element. *Mol Cancer Ther* 8:14
- Donate LE, Blasco MA (2011) Telomeres in cancer and ageing. *Philos Trans R Soc Lond B Biol Sci* 366:76–84

- D'Souza RJ, Sheikh ZA, Busund LT, Russell PJ, Crowe PJ, Yang JL (2003) Expression of nm23 protein in adult soft tissue sarcoma is correlated with histological grade. *Anticancer Res* 23:3289–3294
- Egistelli L, Chichiarelli S, Gauci E et al (2009) IFI16 and NM23 bind to a common DNA fragment both in the P53 and the cMYC gene promoters. *J Cell Biochem* 106:666–672
- Engel M, Theisinger B, Seib T et al (1993) High levels of nm23-H1 and nm23-H2 messenger RNA in human squamous-cell lung carcinoma are associated with poor differentiation and advanced tumor stages. *Int J Cancer* 55:375–379
- Fernandez PC, Frank SR, Wang L et al (2003) Genomic targets of the human c-Myc protein. *Genes Dev* 17:1115–1129
- Fournier HN, Dupe-Manet S, Bouvard D et al (2002) Integrin cytoplasmic domain-associated protein 1 α (ICAP- α) interacts directly with the metastasis suppressor nm23-H2, and both proteins are targeted to newly formed cell adhesion sites upon integrin engagement. *J Biol Chem* 277:20895–20902
- Fournier HN, Dupe-Manet S, Bouvard D et al (2005) Nuclear translocation of integrin cytoplasmic domain-associated protein 1 stimulates cellular proliferation. *Mol Biol Cell* 16:1859–1871
- Francois-Moutal L, Marcillat O, Granjon T (2014) Structural comparison of highly similar nucleoside-diphosphate kinases: molecular explanation of distinct membrane-binding behavior. *Biochimie* 105:110–118
- Freije JM, Blay P, MacDonald NJ, Manrow RE, Steeg PS (1997) Site-directed mutation of Nm23-H1 mutations lacking motility suppressive capacity upon transfection are deficient in histidine-dependent protein phosphotransferase pathways in vitro. *J Biol Chem* 272:5525–5532
- Fukuda M, Ishii A, Yasutomo Y et al (1996) Decreased expression of nucleoside diphosphate kinase alpha isoform, an nm23-H2 gene homolog, is associated with metastatic potential of rat mammary-adenocarcinoma cells. *Int J Cancer* 65:531–537
- Garcia I, Mayol G, Rios J et al (2012) A three-gene expression signature model for risk stratification of patients with neuroblastoma. *Clin Cancer Res* 18:2012–2023
- Gervasi F, D'Agnano I, Vossio S, Zupi G, Sacchi A, Lombardi D (1996) Nm23 influences proliferation and differentiation of PC12 cells in response to nerve growth factor. *Cell Growth Differ* 7:1689–1695
- Gilles AM, Presecan E, Vonica A, Lascu I (1991) Nucleoside diphosphate kinase from human erythrocytes structural characterization of the two polypeptide chains responsible for heterogeneity of the hexameric enzyme. *J Biol Chem* 266:8784–8789
- Gordon GJ, Rockwell GN, Jensen RV et al (2005) Identification of novel candidate oncogenes and tumor suppressors in malignant pleural mesothelioma using large-scale transcriptional profiling. *Am J Pathol* 166:1827–1840
- Goueli BS, Janknecht R (2004) Upregulation of the catalytic telomerase subunit by the transcription factor ER81 and oncogenic HER2/Neu, Ras, or Raf. *Mol Cell Biol* 24:25–35
- Hailat N, Keim DR, Melhem RF et al (1991) High levels of p19/nm23 protein in neuroblastoma are associated with advanced stage disease and with N-myc gene amplification. *J Clin Invest* 88:341–345
- Haitel A, Wiener HG, Baethge U, Marberger M, Susani M (2000) Mdm2 expression as a prognostic indicator in clear cell renal cell carcinoma: comparison with p53 overexpression and clinicopathological parameters. *Clin Cancer Res* 6:1840–1844
- Hamy CV, Abbi R, Prasad N et al (2000) Expression of a catalytically inactive H118Y mutant of nm23-H2 suppresses the metastatic potential of line IV C1 1 human melanoma cells. *Int J Cancer* 88:547–553
- Haring J, Schuler S, Latratch C, Ortmann O, Treeck O (2012) Role of estrogen receptor beta in gynecological cancer. *Gynecol Oncol* 127:673–676
- Hartsough MT, Morrison DK, Salemo M et al (2002) Nm23-H1 metastasis suppressor phosphorylation of kinase suppressor of Ras via a histidine protein kinase pathway. *J Biol Chem* 277:32389–32399
- Herak Bosnar M, Bago R, Konjevoda P, Pavelic J (2008) Gene expression profiling of Nm23-H2 overexpressing CAL 27 cells using DNA microarray. *Neoplasma* 55:447–454
- Hikita ST, Kosik KS, Clegg DO, Bamdad C (2008) MUC1* mediates the growth of human pluripotent stem cells. *PLoS One* 3:e3312
- Hippe HJ, Wolf NM, Abu-Taha HI et al (2011) Nucleoside diphosphate kinase B is required for the formation of heterotrimeric G protein containing caveolae. *Naunyn Schmiedebergs Arch Pharmacol* 384:461–472
- Hudelist G, Czerwenka K, Singer C, Pischinger K, Kubista E, Manavi M (2005) cDNA array analysis of cytobrush-collected normal and malignant cervical epithelial cells: a feasibility study. *Cancer Genet Cytogenet* 158:35–42
- Huwer H, Kalweit G, Engel M, Welter C, Dooley S, Gams E (1997) Expression of the candidate tumor suppressor gene nm23 in the bronchial system of patients with squamous cell lung cancer. *Eur J Cardiothorac Surg* 11:206–209
- Iizuka N, Mori N, Tamesa T, Tangoku A, Oka M (2003) Telomerase activity and Nm23-H2 protein expression in hepatocellular carcinoma. *Anticancer Res* 23:43–47
- Iizuka N, Tsunedomi R, Tamesa T et al (2006) Involvement of c-myc-regulated genes in hepatocellular carcinoma related to genotype-C hepatitis B virus. *J Cancer Res Clin Oncol* 132:473–481
- Iwashita S, Fujii M, Mukai H, Ono Y, Miyamoto M (2004) Lbc proto-oncogene product binds to and could be negatively regulated by metastasis suppressor nm23-H2. *Biochem Biophys Res Commun* 320:1063–1068
- Joosten M, Blazquez-Domingo M, Lindeboom F et al (2004) Translational control of putative protooncogene Nm23-M2 by cytokines via phosphoinositide 3-kinase signaling. *J Biol Chem* 279:38169–38176
- Kang Y, Lee DC, Han J et al (2007) NM23-H2 involves in negative regulation of Diva and Bcl2L10 in apoptosis signaling. *Biochem Biophys Res Commun* 359:76–82
- Kar A, Saha D, Purohit G et al (2012) Metastases suppressor NME2 associates with telomere ends and telomerase and reduces telomerase activity within cells. *Nucleic Acids Res* 40:2554–2565
- Lacombe ML, Milon L, Munier A, Mehuis JG, Lambeth DO (2000) The human Nm23/nucleoside diphosphate kinases. *J Bioenerg Biomembr* 32:247–258
- Lee MJ, Xu DY, Li H et al (2012) Pro-oncogenic potential of NM23-H2 in hepatocellular carcinoma. *Exp Mol Med* 44:214–224
- Lee MY, Jeong WJ, Oh JW, Choi KY (2009) NM23H2 inhibits EGF- and Ras-induced proliferation of NIH3T3 cells by blocking the ERK pathway. *Cancer Lett* 275:221–226
- Lim JQ, Lu J, He BP (2012) Diva/BclB regulates differentiation by inhibiting NDPKB/Nm23H2-mediated neuronal differentiation in PC-12 cells. *BMC Neurosci* 13:123
- MacDonald NJ, De la Rosa A, Benedict MA, Freije JM, Krutsch H, Steeg PS (1993) A serine phosphorylation of Nm23, and not its nucleoside diphosphate kinase activity, correlates with suppression of tumor metastatic potential. *J Biol Chem* 268:25780–25789
- Mahanta S, Fessler SP, Park J, Bamdad C (2008) A minimal fragment of MUC1 mediates growth of cancer cells. *PLoS One* 3:e2054
- Maida Y, Kyo S, Kanaya T et al (2002) Direct activation of telomerase by EGF through Ets-mediated transactivation of TERT via MAP kinase signaling pathway. *Oncogene* 21:4071–4079
- Manavi M, Hudelist G, Fink-Retter A, Gschwandter-Kaulich D, Pischinger K, Czerwenka K (2007) Gene profiling in Pap-cell smears of high-risk human papillomavirus-positive squamous cervical carcinoma. *Gynecol Oncol* 105:418–426
- Mandai M, Konishi I, Komatsu T et al (1995) Mutation of the nm23 gene, loss of heterozygosity at the nm23 locus and K-ras mutation in ovarian carcinoma: correlation with tumour progression and nm23 gene expression. *Br J Cancer* 72:691–695

- Marone M, Scambia G, Ferrandina G et al (1996) Nm23 expression in endometrial and cervical cancer: inverse correlation with lymph node involvement and myometrial invasion. *Br J Cancer* 74:1063–1068
- Martinez JA, Prevot S, Nordlinger B et al (1995) Overexpression of nm23-H1 and nm23-H2 genes in colorectal carcinomas and loss of nm23-H1 expression in advanced tumour stages. *Gut* 37:712–720
- Masoudi N, Fancsalszky L, Pourkarimi E et al (2013) The NM23-H1/H2 homolog NDK-1 is required for full activation of Ras signaling in *C. elegans*. *Development* 140:3486–3495
- Mileo AM, Piombino E, Severino A, Tritarelli A, Paggi MG, Lombardi D (2006) Multiple interference of the human papillomavirus-16 E7 oncoprotein with the functional role of the metastasis suppressor Nm23-H1 protein. *J Bioenerg Biomembr* 38:215–225
- Miyamoto M, Iwashita S, Yamaguchi S, Ono Y (2009) Role of nm23 in the regulation of cell shape and migration via Rho family GTPase signals. *Mol Cell Biochem* 329:175–179
- Miyazaki H, Fukuda M, Ishijima Y et al (1999) Overexpression of nm23-H2/NDP kinase B in a human oral squamous cell carcinoma cell line results in reduced metastasis, differentiated phenotype in the metastatic site, and growth factor-independent proliferative activity in culture. *Clin Cancer Res* 5:4301–4307
- Monig SP, Nolden B, Lubke T et al (2007) Clinical significance of nm23 gene expression in gastric cancer. *Anticancer Res* 27:3029–3033
- Murakami H, Sanderson ND, Nagy P, Marino PA, Merlino G, Thorgeirsson SS (1993) Transgenic mouse model for synergistic effects of nuclear oncogenes and growth factors in tumorigenesis: interaction of c-myc and transforming growth factor alpha in hepatic oncogenesis. *Cancer Res* 53:1719–1723
- Neidle S (2010) Human telomeric G-quadruplex: the current status of telomeric G-quadruplexes as therapeutic targets in human cancer. *FEBS J* 277:1118–1125
- New DC, Wong YH (2007) Molecular mechanisms mediating the G protein-coupled receptor regulation of cell cycle progression. *J Mol Signal* 2:2. doi:10.1186/1750-2187-2-2
- Niitsu N, Okabe-Kado J, Nakayama M et al (2000) Plasma levels of the differentiation inhibitory factor nm23-H1 protein and their clinical implications in acute myelogenous leukemia. *Blood* 96:1080–1086
- Niitsu N, Okabe-Kado J, Okamoto M et al (2001) Serum nm23-H1 protein as a prognostic factor in aggressive non-Hodgkin lymphoma. *Blood* 97:1202–1210
- Nosaka K, Kawahara M, Masuda M, Satomi Y, Nishino H (1998) Association of nucleoside diphosphate kinase nm23-H2 with human telomeres. *Biochem Biophys Res Commun* 243:342–348
- Ohya S, Fukuyo Y, Kito H et al (2014) Upregulation of KCa3.1 K⁺ channel in mesenteric lymph node CD4⁺ T lymphocytes from a mouse model of dextran sodium sulfate-induced inflammatory bowel disease. *Am J Physiol Gastrointest Liver Physiol* 306:G873–G885
- Okabe-Kado J, Kasukabe T, Honma Y, Hayashi M, Henzel WJ, Hozumi M (1992) Identity of a differentiation inhibiting factor for mouse myeloid leukemia cells with NM23/nucleoside diphosphate kinase. *Biochem Biophys Res Commun* 182:987–994
- Okabe-Kado J, Kasukabe T, Baba H, Urano T, Shiku H, Honma Y (1995a) Inhibitory action of nm23 proteins on induction of erythroid differentiation of human leukemia cells. *Biochim Biophys Acta* 1267:101–106
- Okabe-Kado J, Kasukabe T, Hozumi M et al (1995b) A new function of Nm23/NDP kinase as a differentiation inhibitory factor, which does not require its kinase activity. *FEBS Lett* 363:311–315
- Okabe-Kado J, Kasukabe T, Honma Y (1998) Differentiation inhibitory factor Nm23 as a prognostic factor for acute myeloid leukemia. *Leuk Lymphoma* 32:19–28
- Okabe-Kado J, Kasukabe T, Honma Y (2002) Expression of cell surface NM23 proteins of human leukemia cell lines of various cellular lineage and differentiation stages. *Leuk Res* 26:569–576
- Okabe-Kado J, Kasukabe T (2003) Physiological and pathological relevance of extracellular NM23/NDP kinases. *J Bioenerg Biomembr* 35:89–93
- Paravicini G, Steinmayr M, Andre E, Becker-Andre M (1996) The metastasis suppressor candidate nucleotide diphosphate kinase NM23 specifically interacts with members of the ROR/RZR nuclear orphan receptor subfamily. *Biochem Biophys Res Commun* 227:82–87
- Polanski R, Maguire M, Nield PC et al (2011) MDM2 interacts with NME2 (non-metastatic cells 2, protein) and suppresses the ability of NME2 to negatively regulate cell motility. *Carcinogenesis* 32:1133–1142
- Postel EH, Berberich SJ, Flint SJ, Ferrone CA (1993) Human c-myc transcription factor PuF identified as nm23-H2 nucleoside diphosphate kinase, a candidate suppressor of tumor metastasis. *Science* 261:478–480
- Postel EH, Weiss VH, Beneken J, Kirtane A (1996) Mutational analysis of NM23-H2/NDP kinase identifies the structural domains critical to recognition of a c-myc regulatory element. *Proc Natl Acad Sci U S A* 93:6892–6897
- Postel EH, Abramczyk BM, Levit MN, Kyin S (2000) Catalysis of DNA cleavage and nucleoside triphosphate synthesis by NM23-H2/NDP kinase share an active site that implies a DNA repair function. *Proc Natl Acad Sci U S A* 97:14194–14199
- Postel EH, Abramczyk BA, Gursky SK, Xu Y (2002) Structure-based mutational and functional analysis identify human NM23-H2 as a multifunctional enzyme. *Biochemistry* 41:6330–6337
- Postel EH, Zou X, Notterman DA, La Perle KMD (2009) Double knock-out Nme1/Nme2 mouse model suggests a critical role for NDP kinases in erythroid development. *Mol Cell Biochem* 329:45–50
- Radovic S, Doric M, Hukic A, Babic M, Kusunovic S, Spahovic N (2013) Immunohistochemical expression and significance of NM23 suppressor protein in primary gastric adenocarcinoma. *Bosn J Basic Med Sci* 13:72–77
- Rayner K, Chen YX, Hibbert B et al (2007) NM23-H2, an estrogen receptor β -associated protein, shows diminished expression with progression of atherosclerosis. *Am J Physiol Regul Integr Comp Physiol* 292:R743–R750
- Rayner K, Chen YX, Hibbert B et al (2008) Discovery of NM23-H2 as an estrogen receptor beta-associated protein: role in estrogen-induced gene transcription and cell migration. *J Steroid Biochem Mol Biol* 108:72–81
- Rochdi MD, Laroche G, Dupre E et al (2004) Nm23-H2 interacts with a G protein-coupled receptor to regulate its endocytosis through an Rac1-dependent mechanism. *J Biol Chem* 279:18981–18989
- Rumjahn SM, Javed MA, Wong N, Law WE, Buxton IL (2007) Purinergic regulation of angiogenesis by human breast carcinoma-secreted nucleoside diphosphate kinase. *Br J Cancer* 97:1372–1380
- Sarris M, Lee CS (2001) nm23 protein expression in colorectal carcinoma metastasis in regional lymph nodes and the liver. *Eur J Surg Oncol* 27:170–174
- Sato Y, Tsuchiya B, Urao T, Baba H, Shiku H, Kodama T, Kameya T (2000) Semiquantitative immunoblot analysis of nm23-H1 and -H2 isoforms in adenocarcinomas of the lung: prognostic significance. *Pathol Int* 50:200–205
- Shaul YD, Seger R (2007) The MEK/ERK cascade: from signaling specificity to diverse functions. *Biochim Biophys Acta* 1773:1213–1226
- Smagge BJ, Stewart AK, Carter MG et al (2013) MUC1* ligand, NM23-H1, is a novel growth factor that maintains human stem cells in a more naive state. *PLoS One* 8:e58601
- Srivastava S, Li Z, Ko K et al (2006) Histidine phosphorylation of the potassium channel KCa3.1 by nucleoside diphosphate kinase B is required for activation of KCa3.1 and CD4 T cells. *Mol Cell* 24:665–675
- Steege PS (1991) Genetic control of the metastatic phenotype. *Semin Cancer Biol* 2:105–110

- Steeg PS, Bevilacqua G, Kopper L et al (1988) Evidence for a novel gene associated with low tumor metastatic potential. *J Natl Cancer Inst* 80:200–204
- Steeg PS, de la Rosa A, Flatow U, MacDonald NJ, Benedict M, Leone A (1993) Nm23 and breast cancer metastasis. *Breast Cancer Res Treat* 25:175–187
- Steeg PS, Zollo M, Wieland T (2011) A critical evaluation of biochemical activities reported for the nucleoside diphosphate kinase/Nm23/Awd family proteins: opportunities and missteps in understanding their biological functions. *Naunyn Schmiedeberg's Arch Pharmacol* 384:331–339
- Syed V, Mukherjee K, Lyons-Weiler J et al (2005) Identification of ATF-3, caveolin-1, DLC-1, and NM23-H2 as putative antitumorigenic, progesterone-regulated genes for ovarian cancer cells by gene profiling. *Oncogene* 24:1774–1787
- Tagashira H, Hamazaki K, Tanaka N, Gao C, Namba M (1998) Reduced metastatic potential and c-myc overexpression of colon adenocarcinoma cells (Colon 26 line) transfected with nm23-R2/rat nucleoside diphosphate kinase alpha isoform. *Int J Mol Med* 2:65–68
- Tannapfel A, Anhalt K, Hausermann P et al (2003) Identification of novel proteins associated with hepatocellular carcinomas using protein microarrays. *J Pathol* 201:238–249
- Tas F, Tuzlali S, Aydiner A et al (2002) Prognostic role of nm23 gene expression in patients with ovarian cancer. *Am J Clin Oncol* 25:164–167
- Toksoz D, Williams DA (1994) Novel human oncogene lbc detected by transfection with distinct homology regions to signal transduction products. *Oncogene* 9:621–628
- Tschiedel S, Gentilini C, Lange T et al (2008) Identification of NM23-H2 as a tumour-associated antigen in chronic myeloid leukaemia. *Leukemia* 22:1542–1550
- Tschiedel S, Bach E, Jilo A et al (2012) Bcr-Abl dependent post-transcriptional activation of NME2 expression is a specific and common feature of chronic myeloid leukemia. *Leuk Lymphoma* 53:1569–1576
- Tso PH, Wang YC, Yung LY, Tong Y, Lee MMK, Wong YH (2013) RGS19 inhibits Ras signaling through Nm23H1/2-mediated phosphorylation of the kinase suppressor of Ras. *Cell Signal* 25:1064–1074
- Urano T, Takamiya K, Furukawa K, Shiku H (1992) Molecular cloning and functional expression of the second mouse nm23/NDP kinase gene, nm23-M2. *FEBS Lett* 309:358–362
- Valentijn LJ, Koppen A, van Asperen R et al (2005) Inhibition of a new differentiation pathway in neuroblastoma by copy number defects of N-myc, Cdc42, and nm23 genes. *Cancer Res* 65:3136–3145
- van Steensel B, de Lange T (1997) Control of telomere length by the human telomeric protein TRF1. *Nature* 385:740–743
- Webb PA, Perisic O, Mendola CE, Backer JM, Williams RL (1995) The crystal structure of a human nucleoside diphosphate kinase, NM23-H2. *J Mol Biol* 251:574–587
- Wei SJ, Trempus CS, Ali RC, Hansen LA, Tennant RW (2004) 12-O-tetradecanoylphorbol-13-acetate and UV radiation-induced nucleoside diphosphate protein kinase B mediates neoplastic transformation of epidermal cells. *J Biol Chem* 279:5993–6004
- Wieland T (2007) Interaction of nucleoside diphosphate kinase B with heterotrimeric G protein betagamma dimers: consequences on G protein activation and stability. *Naunyn Schmiedeberg's Arch Pharmacol* 374:373–383
- Willems R, Van Bockstaele DR, Lardon F et al (1998) Decrease in nucleoside diphosphate kinase (NDPK/nm23) expression during hematopoietic maturation. *J Biol Chem* 273:13663–13668
- Willems R, Slegers H, Rodrigus I et al (2002) Extracellular nucleoside diphosphate kinase NM23/NDPK modulates normal hematopoietic differentiation. *Expt Hematol* 30:640–648
- Wuelling M, Delling G, Kaiser E (2004) Differential gene expression in stromal cells of human giant cell tumor of bone. *Virchows Arch* 445:621–630
- Xu JD, Cao XX, Long ZW et al (2011) BCL2L10 protein regulates apoptosis/proliferation through differential pathways in gastric cancer cells. *J Pathol* 223:400–409
- Yamashiro S, Urano T, Shiku H, Furukawa K (1994) Alteration of nm23 gene expression during the induced differentiation of human leukemia cell lines. *Oncogene* 9:2461–2468
- Yao Y, Li C, Zhou X et al. (2014) PIWIL2 induces c-Myc expression by interacting with NME2 and regulates c-Myc-mediated tumor cell proliferation. *Oncotarget* 5:8466–8477
- Yokoyama A, Okabe-Kado J, Sakashita A et al (1996) Differentiation inhibitory factor nm23 as a new prognostic factor in acute monocytic leukemia. *Blood* 88:3555–3561
- Yung LY, Lam WS, Ho MK et al (2012) Astragaloside IV and cycloastragenol stimulate the phosphorylation of extracellular signal-regulated protein kinase in multiple cell types. *Planta Medica* 78:115–121
- Zhu S, Wurdak H, Wang J et al (2009) A small molecule primes embryonic stem cells for differentiation. *Cell Stem Cell* 4:416–426