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Regulation of the metastasis suppressor Nm23-H1 by tumor viruses

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Abstract

Metastasis is the most common cause of cancer mortality. To increase the survival of patients, it is necessary to develop more effective methods for treating as well as preventing metastatic diseases. Recent advancement of knowledge in cancer metastasis provides the basis for development of targeted molecular therapeutics aimed at the tumor cell or its interaction with the host microenvironment. Metastasis suppressor genes (MSGs) are promising targets for inhibition of the metastasis process. During the past decade, functional significance of these genes, their regulatory pathways, and related downstream effector molecules have become a major focus of cancer research. Nm23-H1, first in the family of Nm23 human homologues, is a well-characterized, anti-metastatic factor linked with a large number of human malignancies. Mounting evidence to date suggests an important role for Nm23-H1 in reducing virus-induced tumor cell motility and migration. A detailed understanding of the molecular association between oncogenic viral antigens with Nm23-H1 may reveal the underlying mechanisms for tumor virus-associated malignancies. In this review, we will focus on the recent advances to our understanding of the molecular basis of oncogenic virus-induced progression of tumor metastasis by deregulation of Nm23-H1.

Keywords

Nm23-H1; Metastasis; Cancer; Apoptosis; Oncogenic tumor viruses

Introduction

Metastasis and Nm23: a general overview

Metastasis is considered to be the spread of genetically altered malignant cells from the primary neoplasm to distant organs (Gupta and Massague 2006). Neoplasia or cancer produces uncontrolled proliferative cells, and this uncontrolled proliferation creates a primary heterogeneous tumor (Fidler and Hart 1982). The progression of human cancer has been well characterized by the occurrence of several pre-malignant lesions involving metaplasia, followed by dysplasia then anaplasia, resulting in a malignant phenotype. From a clinical and experimental point of view, formation of the primary tumor and onset of tumor

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metastasis are two distinct processes (Yokota 2000). Basically, in a specific site, tumors can progress without the development of metastasis. A small number of tumorigenic cells can accumulate the full complement of alterations which enables them to disseminate from the primary tumor site. Eventually, they survive from immune system detection as well as biophysical forces and develop tumors at new sites after responding to growth-promoting or growth-inhibitory signals from the distant tissues (Rinker-Schaeffer et al. 2006). Several in-depth studies have identified sets of genes and proteins that can specifically prevent cellular metastasis. These “metastasis suppressors” are providing new insights into our understanding of the intricate molecular mechanisms which regulate progression of tumor metastasis.

Metastasis suppressors which include Nm23-H1, KISS1, RECK, RhoGDI2, MKK6, MKK4, MKK7, and SSeCKS can exert their anti-metastatic activities in several ways by signal transduction (Horak et al. 2008). Interestingly, metastasis suppressors expressing cells grow at primary sites, but they fail to multiply at the secondary site, which suggests differential responses to the site-specific external signals (Rinker-Schaeffer et al. 2006). The discovery of the first metastasis suppressor gene Nm23-H1 provided vital functional evidence for the existence of genes which specifically regulate tumor metastasis (Steeg et al. 1988). Rosengard et al. identified the nm23-H1 gene, for which reduced RNA levels were observed in tumor cells of high metastatic potential (Rosengard et al. 1989). A second human gene, Nm23-H2, was identified by its high homology with Nm23-H1 (Stahl et al. 1991). Several reports have suggested that expression of Nm23-H1 is inversely proportional to the aggressive metastatic behavior of melanoma and gastric, colon, and breast carcinoma (Kapitanovic et al. 2004). Low levels of nm23-H1 expression have been linked to advanced stage of ovarian carcinoma and metastasis in lymph nodes (Mandai et al. 1994). Interestingly, enhanced nm23-H1 expression was found to correlate with a significant reduction in survival rate for neuroblastoma and osteosarcoma patients (Leone et al. 1993b). Another study by Szumilo et al. suggested that the status of Nm23-H1 is not connected with metastatic ability and prognosis in esophageal squamous cell carcinoma (Szumilo et al. 2002). These studies suggested that Nm23-H1 may possess a tissue-specific role in the context of its contribution to pathogenesis in cancers and a diverse set of regulatory mechanisms. Therefore, it is important to understand the detailed functional role of Nm23-H1 to develop novel therapeutic approaches in the field of cancer invasion and metastasis.

Role of infectious agents in metastasis of human cancers

Cancer or malignant neoplasia can be defined as a group of diseases which involves the uncontrolled proliferation of cells. Ultimately, malignant tumors invade nearby normal tissues and can spread to more distant organs of the body through the circulatory system. The causes of cancer are complex and diverse and continue to be investigated. Several factors are associated with increasing risks of cancer, including use of tobacco, dietary factors and food habits, certain infections, exposure to radiation, lack of physical activities, obesity, and environmental pollutants (Anand et al. 2008). These factors have direct roles in damaging genes or combined with existing genetic faults within cells can cause malignant mutations (Loeb and Loeb 2000). Interestingly, 5–10 % of cancers were characterized by inherited genetic defects (Nagy et al. 2004). Several infectious agents, especially viruses, are

major contributors to a wide range of human malignancies. Viral-mediated infections are directly or indirectly related to one fifth of the cancer population worldwide (Vandeven and Nghiem 2014). Many prominent oncogenic viruses, such as Epstein–Barr virus, human papilloma virus, hepatitis B virus, and human herpesvirus-8, are major contributory factors in progression of human cancers. Human T lymphotropic virus type 1 and hepatitis C viruses are the two RNA viruses that are known contributors to human cancers (Liao 2006; Pattle and Farrell 2006). However, virus infection alone may not be sufficient to drive the progression of cancer. Additional events as well as host factors, including immunosuppression, somatic mutations, genetic predisposition, and exposure to carcinogens, all have important contributory roles (Liao 2006). The main cancer-causing viruses are human papillomaviruses, human polyomaviruses, Epstein–Barr virus (EBV), Kaposi's sarcoma-associated herpesvirus (KSHV), hepatitis B virus (HBV), and hepatitis C virus (HCV), and human T cell leukemia virus-1 (HTLV-1). They have major roles in progression of cervical cancers, mesotheliomas, B cell lymphoproliferative diseases, Kaposi's sarcoma, primary effusion lymphomas, hepatocellular carcinoma, and T cell leukemias, respectively (Pagano et al. 2004). Several efforts have been made to understand the influence of bacterial infection in progression of oncogenesis. Bacteria may also be important contributors to cell transformation directly through the production of different toxic and carcinogenic metabolites (Chang and Parsonnet 2010). Interestingly, some bacteria may also have important roles in cancer progression, such as *Helicobacter pylori* which is linked to gastric carcinoma (Egi et al. 2007) and MALT lymphoma (Morgner et al. 2000) but, in some cases, may also protect against esophageal cancer (Mager 2006). Chronic inflammatory syndrome was observed with chronic *H. pylori* infection in the human stomach. Development of chronic gastritis, atrophic gastritis, intestinal metaplasia, and dysplasia may have influential roles in the pathogenesis of gastric adenocarcinoma (Chang and Parsonnet 2010). Few *H. pylori*-infected cases have actually been shown to lead to the development of gastric cancer. However, some *H. pylori* strains possess a functional cytotoxin-associated gene (cag) pathogenicity island (PAI) (Blaser and Atherton 2004). *H. pylori* was found to insert the CagA protein into the host cell with the help of PAI-encoded type IV secretion system (Shaffer et al. 2011). Consequently, this process ultimately increased the transcription of host genes, changed host cell morphology, and increased inflammatory response which are considered as the higher risk factors for developing gastric adenocarcinoma (Hatakeyama 2006). Additionally, CagA-positive *H. pylori* strains were found to induce the activation of c-fos and c-jun proto-oncogenes (Meyer-ter-Vehn et al. 2000). The etiologic relationship was established between MALT lymphoma and *H. pylori* infection, and immunohistochemical studies revealed the existence of *H. pylori* in 92% of MALT lymphoma tissue samples, even the frequency was found much higher than the typical 50 to 60 % background prevalence of infection (Wotherspoon et al. 1991). Other bacteria including *Salmonella typhi* were found to be associated with gallbladder cancer (Shukla et al. 2000), and *Chlamydia pneumonia* was related with lung cancer (Kocazeybek 2003). *Mycoplasma* may also have a role in progression of different cancers (Namiki et al. 2009).

Cancer patients are highly susceptible to infections with different types of pathogens, such as bacteria, viruses, fungi, and parasites, and inflammation is considered to be one of the

major contributors to the death of cancer patients (Ambrus et al. 1975). Cancer patients have shown the existence of acute pneumonia in 47 % of the cases (Szentirmay et al. 1977). Interestingly, *Streptococcus pneumoniae* was found to be associated indirectly for increasing adhesion, migration, and metastasis of cancer cells (Jedrzejewski 2001). One study by Yan et al. showed that both bacteria and LPS-induced acute lung injury and inflammation were significant causal factors for enhancing lung metastasis (Yan et al. 2013).

Role of tumor viruses in regulation of cellular invasion and metastasis

Several studies have suggested a role for viruses in evading the immune system through cell-to-cell transmission (Parris 2005). Interestingly, there are three modifications that are required for successful cell-to-cell transmission of the host and target cell: (1) The infected cell is induced to express surface adhesion molecules that expedite the fusion of the cell membranes (Cocchi et al. 2000); (2) fusion of the cytoplasm of cells that would normally trigger apoptosis of the cells by p53-dependent mechanisms and so to avoid apoptosis, the viruses would induce overexpression of the host antiapoptotic proteins, Bcl-2, or a viral homologue vBcl-2 (Sarid et al. 1997); (3) unsynchronized mitosis of nuclei would lead to death of the syncytia by the process of “mitotic catastrophe” if normal apoptosis is suppressed (Castedo et al. 2004).

Recently, intensive studies have been carried out to examine the genes and gene products which are responsible for driving the metastatic process. Several groups have revealed that many transcription factors can program many of the cell biological changes needed to perform the initial steps of the invasion–metastasis cascade (Batlle et al. 2000; Comijn et al. 2001). Metastasis suppressor genes consist of a group of genes having potential activity in metastatic colonization that may be amenable to therapeutic development (Horak et al. 2008). The first discovered metastasis suppressor gene was nm23-H1 (Steeg et al. 1988). In a screen for genes which were differentially expressed between tumorigenic, metastatic murine melanoma cell lines and related tumorigenic non-metastatic lines, reduced Nm23-H1 expression was found in the highly metastatic samples (Horak et al. 2008). Various studies suggested that low Nm23-H1 expression level in human tumors often correlated with poor patient survival (Besant and Attwood 2005). However, Nm23-H1 is not yet reported as an independent prognostic factor, and it was reported that transient expression of Nm23-H1 into K-1735 melanoma cells reduced their in vivo metastatic ability by 52 to 96 %, with no effect on the primary tumor size (Stahl et al. 1991). Similar tendencies were observed with breast, colon, oral, and hepatocellular carcinoma and melanoma cell lines in transfection assays (Tagashira et al. 1998). Interestingly, it was observed that forced expression of Nm23-H1 in ovarian carcinoma cells significantly extended mouse survival (Li et al. 2006).

The EBV nuclear protein, EBNA3C, interacts with Nm23-H1, which results in enhanced transactivating activity (Subramanian and Robertson 2002). Both latent EBV proteins (EBNA1 and EBNA3C) bind with Nm23-H1 and inactivate its motility-suppressive effects (Kaul et al. 2009). The expression of EBV nuclear antigens 1 and 3C in MDA-MB-231 human breast carcinoma cells increased ascites and lung metastases in vivo, which was reversed by Nm23-H1 overexpression (Kaul et al. 2009). Similarly, other report suggested

that human papilloma virus oncoprotein E7 binds with Nm23-H1 and regulates tumor cell motility (Mileo et al. 2006).

Among the other EBV oncoproteins, latent membrane protein 1 (LMP1) has the ability to induce invasiveness and metastasis. LMP1 induces matrix metalloproteinase 9 (MMP-9), a type IV collagenase which can disrupt basement membranes (Horikawa et al. 2000). Also, cyclooxygenase-2 (COX-2) is induced by LMP1 in nasopharyngeal carcinoma (NPC) (Muroño et al. 2001). The I κ B super-repressor and aspirin are considered to be two inhibitors of the NF- κ B signaling pathway. They can reduce the induction of MMP-9 and COX-2 and invasiveness of LMP1-expressing cells. LMP1-induced production of vascular endothelial growth factor (VEGF) is also reduced by a COX-2-specific inhibitor (Muroño et al. 2001). A study by Wakisaka et al. demonstrated that LMP1 induces expression of the angiogenic fibroblast growth factor-2 (FGF-2) (Wakisaka and Pagano 2003). Furthermore, the expression level of LMP2A correlates with expression levels of cellular integrin alpha 6 (ITG α 6) in NPC biopsy samples suggesting a role in cellular migration and metastasis. In this study, LMP2A expression in primary tonsil epithelial cells induced migration and invasiveness of the tumor cells (Pegtel et al. 2005). Moreover, higher transcript levels of ITG α 6 were found in LMP2-expressing epithelial cells as well as an increase in protein level. This studies provided a connection between LMP2A expression, ITG α 6 expression, epithelial cell migration, and NPC metastasis, suggesting a contribution by EBV to the high metastatic incidence of NPC (Pegtel et al. 2005).

Hepatocellular carcinoma (HCC) is a highly aggressive carcinoma of the liver and is the third most common cause of cancer-related death worldwide. HCC is a complex and heterogeneous tumor with frequent intrahepatic spread and extrahepatic metastasis (Llovet and Bruix 2008). HCC development rates among hepatitis C virus (HCV)-infected persons were found to range from 1 to 4 %. Hepatocarcinogenesis is considered a multistep process involving uncontrolled cellular proliferation, detachment from the extracellular matrix (ECM), and invasion into the surrounding tissue, along with modulation of both the immune and circulatory systems for tumor progression (Di Bisceglie et al. 2003). Osteopontin (OPN) is a secreted phosphoprotein which has been linked to tumor progression and metastasis in a variety of cancers (Rangaswami et al. 2006). Iqbal et al. demonstrated the role of HCV-induced OPN in epithelial to mesenchymal transition (EMT), hepatoma cell migration, and invasion (Iqbal et al. 2014). Their study also revealed that HCV-induced OPN interacts with integrin α V β 3 as well as CD44 at the cell surface and induces signal transduction through the phosphorylation of FAK, Akt, and Src. Taken together, these observations provided new insights into the role of OPN activation and secretion in EMT and migration and invasion of human hepatocytes related with chronic HCV infection (Iqbal et al. 2014).

Kaposi's sarcoma-associated herpesvirus (KSHV) is etiologically linked with Kaposi's sarcoma (KS), the most common AIDS-related malignancy (Zhou et al. 2013). KSHV vIL-6 promotes KS development, but the exact mechanism remains unclear. A study by Wu et al. showed that the KSHV vIL-6 enhanced the expression of DNA methyltransferase 1 (DNMT1) in endothelial cells which increased the global methylation of genomic DNA and promoted cell proliferation and migration (Wu et al. 2014).

In summary, tumor metastasis is a process by which cancer cells migrate from primary tumor site to settle at other sites of the body leading to death of cancer patients. Cancer metastasis involves cellular migration in addition with proteolytic degradation of extracellular matrix (Liotta 1992). Various infectious agents, primarily bacteria and viruses, are major contributors of several human malignancies worldwide. Different bacteria, including *H. pylori*, *C. pneumonia*, and *Mycoplasma*, have important role in progression of neoplasia. Other cancer-causing viruses, including human papillomaviruses and polyomaviruses, EBV, KSHV, HBV, HCV, and HTLV-1, are responsible for contributing up to 20 % of malignancies around the world. Oncogenic viral antigens are expressed by these tumor viruses to deregulate normal cellular functions for accelerating the process of carcinogenesis. During cancer progression, several genes alter their functions to acquire the fundamental microenvironment for metastatic progression, including altered cell adhesion, uncontrolled cell proliferation, increased cellular motility, and increased invasion and anchorage-independent growth. The anti-metastatic Nm23-H1 gene was first identified because its transcript was found reduced in highly metastatic rodent tumor cells (Steeg et al. 1988). Several studies also suggested a vital role of Nm23-H1 in pathogenesis of cancers as well as in different cellular functions. Therefore, it is important to obtain a deeper understanding of the functional role of Nm23-H1 in the field of metastasis.

The relationship between tumor metastasis and Nm23 family members

Nm23 was the first identified metastasis suppressor gene (Steeg et al. 1988). Earlier reports suggested that expression of Nm23-H1 correlates with the histopathological indicators of metastasis, lymph node infiltration, and poor prognosis of several malignancies (Garzia et al. 2006). The nm23 gene family was found highly conserved among a wide range of eukaryotes (Rinker-Schaeffer et al. 2006). This family consists of eight to ten members in mammalian organisms, and Nm23-H9 and Nm23-H10/RP2 were the most recently discovered (Bilitou et al. 2009). Nm23-H1 possesses a strong metastasis suppressor function (Otsuki et al. 2001) and was found to inhibit the metastatic potential of cervical cancer cells (Tee et al. 2006). Enhanced expression of Nm23-H1 in melanoma and breast carcinoma reduces transforming growth factor (TGF)- β 1 cytokine response (Leone et al. 1991, 1993a). As a potential anti-metastatic protein, Nm23-H1 can regulate the transcription of a set of genes which are related to metastasis in lung cancer cell lines (Che et al. 2005) and inhibits the anchorage-dependent growth and extracellular matrix adhesion of prostate cancer cells (Lim et al. 1998). Both nm23-H1 and nm23-H2 have been identified in humans (Stahl et al. 1991), and their encoded protein products were shown to be identical with human NDP kinases A and B, respectively (Gilles et al. 1991). Previous studies suggested important roles of nm23-H1 compared to nm23-H2 in intrahepatic metastasis of hepatocellular carcinoma (Iizuka et al. 1995). Other reports also indicated that Nm23-H2 is less involved in the context of metastasis suppression compared to its homolog Nm23-H1 (Hamby et al. 2000). In contrast, it was also notified that enhanced expression of Nm23-H2 inhibits cell migration and colonization (Syed et al. 2005). Reduced expression of Nm23-H2 was detected in cancer cells with reduced c-myc expression which decreases the rate of apoptosis by enhancing metastasis (Thakur et al. 2009). Another family member Nm23-H3 has important roles in reducing cell motility and correlated with metastatic progression (Carinci et al. 2007).

In summary, the nm23 gene family consists of ten members and is highly conserved among eukaryotes. Several studies suggested that the nm23 gene family is critical for regulating cellular metastasis and invasion. As the first identified metastasis suppressor gene, nm23 expression level was linked to poor prognosis of different human cancers. Among the different family members, Nm23-H1 has potent metastasis suppressor function. Nm23-H1 also regulates the transcriptional activities of multiple genes linked to invasion and metastasis. Another family member Nm23-H2 is less involved in metastasis suppression compared to Nm23-H1. However, enhanced expression of Nm23-H2 prevents cell migration. Furthermore, another family member Nm23-H3 possesses the potentiality to reduce cell motility.

Nm23-H1: a regulator of cellular metastasis, proliferation, differentiation, and apoptosis

The nm23-H1 gene was considered as the first isolated metastasis suppressor gene, localized on chromosome 17q21.3 (Chow et al. 2000). In human malignancies, the clinical relevance of nm23-H1 as a potential metastasis suppressor remains enigmatic. Decreased nm23-H1 expression was reported to be associated with tumor metastasis in a varieties of carcinomas, including liver (Nakayama et al. 1992), breast (Royds et al. 1993), stomach (Kodera et al. 1994), and ovary (Mandai et al. 1994). Nm23-H1 is well known for harboring various enzymatic activities as well as its ability to regulate different cellular functions. Table 1 shows a list of important biological functions of Nm23-H1. Studies with highly metastatic human breast cancer cells by transfection assays support the hypothesis in the context of metastatic potential in vivo, as well as colonization and cell motility in vitro (Howlett et al. 1994). Interestingly, expression levels of Nm23-H1 were demonstrated to be inversely related to the survival of patients with carcinomas of the stomach (Kodera et al. 1994), larynx (Lee et al. 1996), and ovary (Scambia et al. 1996), but not with the carcinomas of the breast (Sastre-Garau et al. 1992), lung (Myeroff and Markowitz 1993), uterine/cervix (Mandai et al. 1995), and kidney (Walther et al. 1995). In contrast, higher expression of Nm23-H1, without any allelic deletion or genetic amplification, was positively related to the progression of thyroid (Zou et al. 1993), pancreatic (Nakamori et al. 1993), and lung cancers (Gazzeri et al. 1996). Not only does Nm23-H1 act as a tumor metastasis suppressor, but it also performs important roles as nucleoside diphosphate kinase by converting nucleoside diphosphates to nucleoside triphosphates with an expense of ATP (Choudhuri et al. 2010). It regulates several cellular activities, including proliferation, development, migration, and differentiation, via modulation of cellular signal transduction pathways. Previous report by Choudhuri et al. suggested that Nm23-H1 may have a role for regulating cell cycle and apoptosis in human B cells (Choudhuri et al. 2010). Other reports showed that knockdown of Nm23-H1 reduced proliferation and increased the percentage of cells arrested in the G0/G1 phase of the cell cycle (Jin et al. 2009). In neoplasia, regulation of normal breast differentiation was found deregulated by modulation of the basement membrane microenvironment. Interestingly, studies have suggested an important role of Nm23-H1 in modulation of cellular responsiveness to the microenvironment (Howlett et al. 1994).

Specific interacting partners of Nm23-H1 are linked to diverse cellular functions

Nm23-H1 possesses the ability to interact with several cellular proteins and modulate a range of important biochemical pathways (Fig. 1). Interestingly, macrophage migration inhibitory factor (MIF) has an important role for regulating host inflammatory and immune response (Calandra and Roger 2003). Higher expression of MIF was observed in human melanoma, breast carcinoma, metastatic prostate cancer, and adenocarcinoma of the lung (del Vecchio et al. 2000; Meyer-Siegler 2000), and it has been shown to augment tumor cell migration (Ogawa et al. 2000). Jung et al. has shown that the physical association of Nm23-H1 and MIF is mediated by cysteine residues on both proteins (Jung et al. 2008). Experimental evidence also suggested that suppression of p53-mediated apoptosis and cell cycle arrest by MIF is alleviated by the direct interaction of Nm23-H1 with MIF (Jung et al. 2008).

Dbl-1, an oncoprotein which is linked to guanine exchange and belongs to a family of guanine exchange factors (GEF), was identified from a diffuse B cell lymphoma DNA library. Identification of onco-Dbl resulted in the cloning of the proto-oncogene pDbl (Ron et al. 1988). Studies by Murakami et al. demonstrated an interaction between Nm23-H1 and the GEF-associated Dbl-1 (Murakami et al. 2008). Moreover, Nm23-H1 was also shown to bind with pDbl (Murakami et al. 2008). Interestingly, these studies suggested that regulation of the GEF activity by Nm23-H1 interferes with Dbl-1/Cdc42 signaling which ultimately alters the status of cell migration (Murakami et al. 2008). In the context of cell motility, one report indicated that Nm23-H1 binds with gelsolin (Marino et al. 2012, 2013), the founding member of a family of actin-binding proteins which is involved in the remodeling of cellular actin filaments, regulating cellular morphology and movement (Sun et al. 1999). Several in vitro studies showed that overexpression of gelsolin stimulated tumor cell motility and invasion through the modulation of different signaling pathways, including EGFR, PI3K, and Ras–PI3K–Rac (Chen et al. 1996; De Corte et al. 2002; Lader et al. 2005). Also, in vivo experiments suggested gelsolin-mediated suppression of epithelial–mesenchymal transition in mammary epithelial cells (Tanaka et al. 2006). Recently, Marino et al. demonstrated that Nm23-H1 binds to gelsolin and abrogates the actin depolymerization activity of gelsolin in vitro (Marino et al. 2012, 2013).

Dynamin is a large GTPase specially required for the fission of clathrin-coated endocytic vesicles from the plasma membrane (Damke et al. 1994). Krishnan et al. demonstrated that nucleoside diphosphate kinase (NDPK), an enzyme encoded by the *Drosophila* abnormal wing discs (*awd*) or human *nm23* genes, controls internalization of synaptic vesicle and dynamin GTPase is required at this stage (Krishnan et al. 2001). Another report suggested that *awd* regulates the levels of FGF receptor and functions synergistically with shi/dynamin during tracheal development (Dammai et al. 2003). Boissan et al. observed that knockdown of NDPKs NM23-H1/H2 repressed dynamin-mediated endocytosis (Boissan et al. 2013, 2014). Previous report suggested a possible regulatory role for Rho family GTPases in cellular motility and metastasis (Bouzahzah et al. 2001). Recent study showed the role of Nm23-H1 as a GTPase-activating protein of the Ras-related GTPase Rad (Zhu et al. 1999).

Several GEFs for the Rho family GTPases were identified, including PIX, Vav, and Tiam1, which have a catalytic Dbl homology domain and convert GTPases from a GDP-bound state to the active GTP-bound state. Vav1 and PIX both can activate Cdc42 and Rac1 (Crespo et al. 1997; Manser et al. 1998). Tiam1, a Rac1-specific nucleotide exchange factor, originally was identified in T-lymphoma cells as an invasion- and metastasis-inducing gene (Habets et al. 1994). A more detailed study revealed that Nm23-H1 was associated with Tiam1 through the interaction with its amino-terminal region (Otsuki et al. 2001).

Serine–threonine kinase receptor-associated protein (STRAP) performs multiple functions including interactions with TGF- β receptor, acting as a positive regulator of PDK1 and a negative regulator of TGF- β signaling by the stabilization of TGF- β receptor and Smad7 (Seong et al. 2005). Other studies also suggested the possible involvement of STRAP in tumorigenesis and development (Anumanthan et al. 2006; Halder et al. 2006). TGF- β signaling has been linked to p53-mediated signaling cross talk in which p53 co-operated with Smads for TGF- β gene responses (Cordenonsi et al. 2003). Jung et al. observed that Nm23-H1 and its interacting partner STRAP may play an important role in regulation of the p53-induced signaling pathway (Jung et al. 2007).

Among the several interacting proteins with Nm23-H1, H-Prune has been well characterized. H-Prune belongs to the phosphoesterase (DHH) protein super-family, and its enhanced expression in colorectal, breast, and gastric cancers associates with the degree of lymph node and distant metastasis (Massidda et al. 2010). H-Prune sequence comprises two domains at the N-terminus such as DHH and DHHA2 domains which are involved in its enzymatic functions. Interestingly, inhibition of its phosphodiesterase activity with dipyrindamole reduces cell motility in breast cancer cell lines (D'Angelo et al. 2004). H-Prune mainly interacts with GSK-3 β and with Nm23-H1 with the C-terminal region (Garzia et al. 2006; Kobayashi et al. 2006). Of note, Nm23-H1/H-Prune interaction is facilitated through casein kinase phosphorylation of Ser120, Ser122, and Ser125 of Nm23-H1 (Garzia et al. 2008). Recent work by Carotenuto et al. showed that Nm23-H1/H-Prune C-terminal interaction is responsible for regulating neuroblastoma tumorigenesis (Carotenuto et al. 2013). Kinase suppressor of Ras (KSR) was first identified in *Caenorhabditis elegans* and *Drosophila melanogaster* as a positive regulator of Ras/mitogen-activated protein kinase (MAPK) signaling (Kornfeld et al. 1995; Therrien et al. 1995). Recent study by Tso et al. suggested that Nm23-H1 can phosphorylate KSR and elevated levels of phosphorylated KSR were identified in the nuclear fractions of 293/RGS19 cells. Moreover, Tso et al. observed that Nm23H1/2 can form complexes with RGS19, Ras, or KSR (Tso et al. 2013). In addition, Masoudi et al. first confirmed the important link between KSR and NDPK in vivo in *C. elegans*. Their study demonstrated that *C. elegans* NDK-1/Nm23-H1 influences differentiation by inducing Ras/MAPK signaling (Masoudi et al. 2013). Further, Nm23-H1, having histidine protein kinase activity (Steege et al. 2003), was found to phosphorylate the Ser392 of the 14-3-3 binding sites of KSR1, through histidine intermediate (Hartsough et al. 2002). Overexpression of Nm23-H1 in MDA-MB-435 breast carcinoma cells caused reduced pMAPK expression levels without affecting total MAPK levels. This suggests a specific role for Nm23-H1 in inhibition of the Ras/MAPK signaling activities through phosphorylation of KSR (Hartsough et al. 2002). Previously, Hsp90 was found as a binding partner of KSR (Takacs-Vellai 2014), and Nm23-H1-transfected MDA-MB-435 cells

showed increased co-immunoprecipitation of Hsp90 with KSR1 (Salerno et al. 2005). Nm23-H1 has also been reported to alter important cellular functions by interacting with a wide range of proteins, including ROR/RZR receptors (Paravicini et al. 1996), centrosomes (Roymans et al. 2001), glyceraldehyde-3-phosphate dehydrogenase (Engel et al. 1998), cytoskeletal proteins (Otero 1997), muscarinic receptors (Otero et al. 1999), heat shock proteins (Barthel and Walker 1999), phytochromes (Choi et al. 1999), phosphatases (Shankar et al. 1995), and menin (Ohkura et al. 2001).

The role of oncogenic viruses in regulation of the metastasis suppressor Nm23-H1

Several recent studies in the field of virology suggested that different viral proteins have critical roles in regulating Nm23-H1 functions in the context of metastasis and cancer progression (Fig. 2).

Role of EBV

EBV is a lymphotropic gammaherpesvirus which predominantly targets B cells and epithelial cells and is linked with several human malignancies, including Burkitt's lymphoma, nasopharyngeal carcinoma (NPC), Hodgkin's disease, AIDS-associated lymphoma, and gastric carcinoma (Kaul et al. 2007; Wang et al. 2012). It was shown that EBV has the potential to transform quiescent B cells into continuously proliferating lymphoblastoid cell lines (LCLs) in vitro (Saha and Robertson 2011). Only 11 EBV genes from the 90 encoded by the virus are expressed in EBV-immortalized LCLs (West 2006). The EBV gene products which are expressed during primary lymphocyte infection include six nuclear antigen proteins (EBNAs) 1, 2, 3A, 3B, and 3C and leader protein (LP); two latent infection integral membrane proteins (LMPs) 1, 2A, and 2B; two small RNAs (EBERs); and BamA rightward transcripts (BARTs) (Carter et al. 2002). Extensive studies by several groups have demonstrated important roles of encoded EBNAs and LMP1 in driving EBV-induced pathogenesis and suggest that a multifaceted molecular interplay is essential for deregulation of normal cellular functions (Maruo et al. 2006).

Role of EBNA1—EBNA1 is expressed in all phases of EBV latency and important for the maintenance of the EBV episome (Imai et al. 1998). As a DNA-binding phosphoprotein, EBNA1 is responsible for tethering the virus episome to host chromosomes (Marechal et al. 1999). Other studies indicate that EBNA1 antisense oligodeoxyribonucleotides repressed proliferation of EBV-immortalized cells and EBNA1 expression in transgenic mice can induce lymphomas (Wilson et al. 1996). Moreover, EBNA1 was found to be responsible for increasing tumorigenic potential and metastatic capability of a NPC cell line (Sheu et al. 1996). Several studies suggested that expression of EBNA1 is associated with regulation of EBV-associated tumors, including EBV-positive NPCs and gastric adenocarcinomas (Murakami et al. 2005).

Detailed study by Murakami et al. showed that EBNA1 acts as an interacting partner with Nm23-H1 (Murakami et al. 2005). They demonstrated that EBNA1 is associated with Nm23-H1 in EBV-infected cells in vitro, as well as in lymphoblastoid cell lines (LCLs).

Moreover, they showed that Nm23-H1 was localized predominantly to the nucleus and colocalizes to similar compartments as EBNA1 in EBV-transformed LCLs. These in vitro experiments further suggested a critical role for EBNA1 in regulating the activities of Nm23-H1 in the context of cell migration (Murakami et al. 2005).

Role of EBNA3C—Epstein–Barr virus (EBV) latent antigen 3C (EBNA3C) was the first identified viral oncoprotein to be associated with Nm23-H1 (Subramanian et al. 2001). EBNA3C was found to reverse the ability of Nm23-H1 to suppress the migration of Burkitt's lymphoma cells and breast carcinoma cells in vitro and so function as a prometastatic agent (Subramanian et al. 2001). Other studies mapped the binding domain for Nm23-H1 as residues 637 to 675 aa of EBNA3C and that this binding region of Nm23-H1 was located within the proline- and glutamine-rich domains which is linked to the transcriptional activation function of EBNA3C (Subramanian and Robertson 2002). This Nm23-H1 binding domain on EBNA3C shows a high degree of sequence homology with the Necdin transcription factor (Matsumoto et al. 2001). Localization of Necdin was observed in the cytoplasm of fully differentiated neurons, and it was found to be translocated to the perinuclear compartments, possibly associating with heterochromatin (Niinobe et al. 2000). Studies by Kaul et al. showed that Nm23-H1 and EBNA3C can modulate the biological functions of Necdin. Their work revealed a new role for EBNA3C in changing the subcellular localization of Necdin as well as rescuing cells from the anti-angiogenic and anti-proliferative effects mediated by Necdin. Moreover, they showed that Necdin is directly associated with Nm23-H1, resulting in modulation of the biochemical function of Nm23-H1, as well as the biological function of Necdin (Kaul et al. 2009).

Role of KSHV

Kaposi's sarcoma-associated herpesvirus (KSHV) is the well-established etiologic agent of Kaposi's sarcoma (KS) (Haque et al. 2003). Despite the reduced incidence of HIV-associated KS in the era of highly active anti-retroviral therapy, KS is still considered to be one of the most common HIV-associated tumors in developing countries and an important cause of morbidity and mortality in patients with solid organ transplants (Li et al. 2006). In addition to KS tumors, KSHV has also been causally associated with two types of lymphoproliferative diseases, primary effusion lymphoma (PEL) and multicentric Castleman's disease (MCD) (Cai et al. 2010b). Attainment of an invasive phenotype represents one of the major hallmarks of KSHV-infected cells and is thought to be a key pathogenic mechanism for the development of the KS phenotype (Ganem 2010). Similar to EBV, KSHV infection is also largely latent and during latency, and only a small subset of viral genes is expressed (Kwun et al. 2007). KSHV-encoded latency-associated nuclear antigen (LANA) is considered as one of the critical genes expressed during latency and is essential for episome persistence in the absence of other virus-encoded genes (Ballestas et al. 1999). In addition to episomal maintenance, LANA functions as a transcriptional modulator of numerous cellular and viral promoters, including its own promoter (Garber et al. 2001). LANA-mediated transcriptional activation was extensively reported to be accompanied by many cellular proteins including ATF, AP-1, CAAT, and SP-1 (Criswell et al. 2003). LANA has also been shown to have transcriptional repressive effects through

interaction with many cellular co-repressors including mSin3, SAP30, CIR, MeCP2, and SUV39H1 (Cai et al. 2010a).

Several lines of evidence support a role for LANA in regulation of many metastasis suppressor genes during KSHV-mediated pathogenesis (Qin et al. 2011). Recently, a more direct function for LANA in connection with metastasis regulation was demonstrated (Qin et al. 2011). The study showed that LANA augments expression levels as well as nuclear translocation of Nm23-H1 and activates Ras–BRAF–MAPK signal transduction pathway, secretion of promigratory factors associated with this pathway, and KSHV-induced cell invasiveness which are all critically dependent on the deregulation of Nm23-H1 activity (Qin et al. 2011). Interestingly, induction of cytoplasmic expression of Nm23-H1 using a pharmacologic inhibitor of DNA methylation showed a marked reduction in activation of KSHV-associated Ras–BRAF–MAPK signaling and cell invasiveness, suggesting a potential therapeutic approach against KSHV-associated human malignancies (Qin et al. 2011). A recent study demonstrated an increase in both total and intranuclear Nm23-H1 expression following de novo KSHV infection and so suggests a direct correlation between intracellular viral load and intranuclear Nm23-H1 expression (Qin et al. 2011). This study also demonstrated intranuclear co-localization of LANA and Nm23-H1. These studies suggest an independent mechanism for LANA translocation of Nm23-H1 and KSHV upregulation of Nm23-H1 expression (Di Bartolo et al. 2008). Some studies also revealed that interaction between LANA and Nm23-H1 may facilitate hypermethylation of the Nm23-H1 promoter, thereby deregulating Nm23-H1. Moreover, LANA-initiated promoter hypermethylation might also explain why the DNA methylation inhibitor 5-aza-2-deoxycytidine reduces KSHV-mediated invasion (Qin et al. 2011). Interestingly, Nm23-H1 is also upregulated by p53 (Rahman-Roblick et al. 2007), and p53 disrupts Nm23-H1 interactions with protein binding partners (Jung et al. 2007).

Previously, it was shown that increased cytoplasmic expression of Nm23-H1 suppressed cell invasiveness induced by KSHV-infection or in presence of LANA (Qin et al. 2011). Studies by Qin et al. also demonstrated that cytoplasmic enhanced expression of Nm23-H1 suppresses Ras–BRAF–MAPK pathway activation and secretion of VEGF and IL-8 by KSHV-infected cells (Qin et al. 2011). This lends further support to Ras–BRAF–MAPK activation of VEGF and IL-8 secretion as a mechanism for KSHV-induced invasiveness (Qin et al. 2011). Studies also determined that invasiveness of cells with enhanced expression of cytoplasmic Nm23-H1 can be partially restored using recombinant human VEGF or rhIL-8 and the expression of Nm23-H1 reduced the ability of conditioned media from KSHV-infected fibroblasts to induce invasiveness in KSHV adolescent endothelial cells. Additionally, an inhibitor of BRAF activation inhibited KSHV-induced secretion of VEGF and IL-8 and invasiveness comparable to that seen with MAPK inhibition (Qin et al. 2011). Furthermore, VEGF and IL-8 were found to be upregulated during KSHV infection (Shin et al. 2008), and previous studies have indicated that the anti-metastatic effects of metastasis suppressor genes are associated with down-regulation of VEGF and other genes associated with cell migration (Su et al. 2006).

One study demonstrated an interaction between DNMT3a and LANA, resulting in methylation and inactivation of its promoter (Shamay et al. 2006). Re-establishment of the

TGF-signaling pathway reduces the viability of KSHV-infected PEL cells and promotes apoptosis (Di Bartolo et al. 2008). It was shown recently that LANA also associates with the promoter of the TGF-type II receptor (TRII) and induces its methylation in PEL cells (Qin et al. 2011). It is further postulated that KSHV infection results in lower expression of TRII in KS and MCD lesions (Parravicini et al. 2000). Another metastasis suppressor gene, p16INK4a, is inactivated by promoter hypermethylation in PEL cells (Qin et al. 2011) and that LANA expression protects lymphoid cells from p16INK4a-induced cell cycle arrest inducing S-phase entry (An et al. 2005). Collectively, these studies established a role for additional metastasis suppressor genes in KS-associated pathogenesis and that regulation of Nm23-H1 by KSHV may synergize with other mechanisms to promote KS progression.

Role of HPV

Human papillomaviruses (HPVs) are linked to human cervical and other associated anogenital cancers (Munoz et al. 2003). Viral genome integration in the transformed epithelial cells is limited to the coding regions for the E6 and E7 oncoproteins (Mileo et al. 2006). E7 plays a major role in cell transformation (Campo-Fernandez et al. 2007). Earlier, the HPV-16-encoded E7 and the interaction with Nm23-H1 and Nm23-H2 proteins were identified by the two-hybrid system and validated by antibody binding assays in human keratinocyte HaCaT cell line (Mileo et al. 2006). E7 oncoprotein expression in HaCaT cells induces modified keratinocyte proliferation and differentiation patterns, and consequently, it downmodulates and functionally inactivates Nm23-H1 protein (Mileo et al. 2006). Both transcriptional down-regulation and protein degradation contributed to reduce Nm23-H1 intracellular levels (Mileo et al. 2006). Along with the metastasis inhibition, Nm23-H1 shows a range of other functions in cell cycle regulation and differentiation, development, DNA regulation, and caspase-independent apoptosis (Mileo et al. 2006). Followed by Nm23-H1 inhibition, HPV-16 E7-expressing HaCaT cells acquire invasiveness capabilities and resistance to granzyme A-induced apoptosis (Vande Broek et al. 2004). Moreover, Nm23-H1 is considered to be a granzyme A-activated DNase during cytotoxic T lymphocyte (CTL)-induced caspase-independent apoptosis of virus infected or transformed cells (Pardo et al. 2004).

Earlier, in vitro studies demonstrated the physical interaction between the E7 protein of high-risk HPVs and Nm23-H1 and suggested that it inhibited the HPV tumorigenic potential (Benevolo et al. 2010). A limited number of clinical studies have been performed to identify the importance of Nm23-H1 as a marker of cervical HPV infection, disease etiology, and the progression of disease (Benevolo et al. 2010). Furthermore, loss of Nm23-H1 might allow HPV-16 E7 to promote cell transformation and tumor progression. There are some contradictions for the specific role of Nm23-H1 in normal and human cervical cancer tissues (Mileo et al. 2006). In vivo studies have observed a significant decrease of Nm23-H1 expression from CIN1 to cancer (Branca et al. 2006).

Role of viruses in modulating the functions of Nm23-H1/Necdin complex

Previous studies identified the amino acids 637 to 675 of EBNA3C as specifically binding to Nm23-H1 flanked by the proline and glutamine-rich domains (Subramanian and Robertson

2002). Blast analysis of this domain sequence identified a homology with transcription factor Necdin (Kaul et al. 2009). Necdin has been associated with genetic inactivation in human Prader–Willi syndrome (PWS) (Lee et al. 2005). The main characteristics of this syndrome are a delay in development, short stature, abnormalities in behavior, the onset of severe obesity in childhood, a consistent drive to eat, referred to as hyperphagia, inadequate ventilation, and reproductive system defects (Bush and Wevrick 2008). Necdin is a member of the MAGE family of proteins, known to have important roles in various cellular processes, including cell cycle regulation and cell death (Gur et al. 2014). The most important feature of all MAGE family proteins is the presence of a large central region which belongs to the MAGE homology domain (MHD) (Kaul et al. 2009). In MHD, the amino- and carboxy-terminal domains are typically shown to be unique for individual family members (Barker and Salehi 2002). Human genes encoding Necdin, MAGE-L2, and MAGE-G1 are located at the proximal chromosome 15q, a region related with neuro-developmental disorders such as PWS, Angelman syndrome, and autistic disorder (Kuwako et al. 2004). The Necdin and MAGE-L2 genes are involved in genomic imprinting and found to be involved in the etiology of PWS (Kuwako et al. 2004). Cell proliferation demonstrated by colony formation and bromodeoxyuridine incorporation assays revealed that necdin and MAGE-G1, but not MAGE-L2, induced growth arrest (Kuwako et al. 2004). Necdin and MAGE-G1 are associated with E2F1 via its transactivation domain, suppressed E2F1-dependent transcription, and antagonized E2F1-induced apoptosis. Additionally, Necdin and MAGE-G1 were found to be interacted with the p75 neurotrophin receptor via its distinct intracellular domains (Kuwako et al. 2004). Contrary to this, MAGE-L2 failed to bind to Necdin-interacting molecules, suggesting that MAGE-L2 is not likely to possess Necdin-like functions. Inducing expression of p75 translocated Necdin and MAGE-G1 to the proximity of the plasma membrane and reduced their binding with E2F1, thus facilitating E2F1-induced death of neuroblastoma cells (Kuwako et al. 2004).

Necdin is a 325aa protein encoded in a cDNA clone isolated from a subtraction library of neurally differentiated mouse embryonal carcinoma cells (Kaul et al. 2009). Ectopic expression of Necdin suppresses the proliferation of several cell lines (Kuwako et al. 2004). Necdin interacts with various cytoplasmic and nuclear proteins (Kobayashi et al. 2002), suggesting that this protein is multifunctional. The localization of Necdin in differentiated neurons was previously demonstrated to show predominantly cytoplasmic signals, with a striking change in location to the nucleus during specific physiological changes (Niinobe et al. 2000). Necdin has also been shown to interact with DNA, which may enable it to regulate transcription of other genes involved in cellular differentiation and proliferation through direct binding with DNA or through its interaction with other transcription factors, including p53 and E2F1 (Maruyama 1996). In metastasis, Necdin can associate with hypoxia-inducible factor 1 (HIF-1), a major transcription regulator in hypoxia, and may directly regulate its activity (Moon et al. 2005). Thus, the regulation of HIF-1 expression by Necdin has important consequences, including downstream effects on cell growth and proliferation.

Kaul et al. observed reduced Necdin transcript levels in EBV-positive lymphoblastoid cells (Kaul et al. 2009). Also, Necdin is known to modify its subcellular distribution under the influence of other proteins, like E2F1 transcription factor and p75NTR, the neurotrophin receptor (Tcherpakov et al. 2002). They reported that Necdin was present in the cytoplasm

when it was expressed alone, but the majority was in the nucleus when co-expressed with EBNA3C (Kaul et al. 2009). They demonstrated that Necdin interacted with membrane proteins which then re-localize to insoluble membrane fractions when expressed in the presence of EBNA3C. Importantly, the amounts of soluble Necdin were similar in the nuclear fraction and the cytoplasm when Necdin was expressed alone and with EBNA3C (Kaul et al. 2009).

EBV-positive LCLs show a significantly higher level of methylation within the CpG sites of the Necdin promoter than do primary lymphocytes (Lau et al. 2004). This suggests that the EBV latent antigens may be involved in regulation of Necdin-mediated functions possibly related to cell cycle regulation and apoptosis. EBV is also known to be associated with a number of human lymphoid and epithelial tumors. EBV type III latency is commonly associated with AIDS-associated lymphomas and post-transplant lymphoproliferative disorders (Rea et al. 1994).

Conclusion

In depth studies of metastasis suppressor genes have continued over the past several decades. The nm23 gene family, a predominant set of regulators in tumor metastasis and invasion, has made advances to prevent cellular metastasis and contributing to a new leading edge of research with potential drug targets (Marshall et al. 2010). Among the family members, the level of Nm23-H1 expression can be used to define the response to treatment following radiotherapy for ovarian cancer and in NPCs (Kapoor 2009). Adenovirus-mediated transfer of nm23-H1 gene to inhibit cellular metastasis can be a developing and an innovative version in therapeutic strategies (Li et al. 2006). Different studies suggested that tumor metastasis is the primary cause of death in cancer patients and modulation of nm23-H1 gene expression can be useful to understand the underlying mechanisms of metastatic invasion in malignant tumors (Marshall et al. 2009). Interestingly, forced expression of Nm23-H1 was found to reduce metastasis of the lungs, lymph node, and other organs. Therefore, Nm23-H1 may serve as an important marker of lymph node metastasis in lung cancer patients, and its expression level can be used as a potential molecular target for cancer therapy (Marshall et al. 2009).

Current insights into the potential of Nm23-H1 to prevent tumor metastasis have shown new therapeutic avenues and possible drug targets to increase treatment opportunities for patients. Importantly, reduced Nm23-H1 expression is associated with the highly aggressive metastasis and with unfavorable clinical prognosis in a wide range of human carcinomas (de la Rosa et al. 1995). Different methods were adopted to restore the anti-metastasis properties of Nm23-H1 as therapeutic strategy, such as Nm23-H1 promoter activation by medroxyprogesterone acetate (MPA) treatment, activation of downstream signaling, and gene target and gene therapeutic approach (Marino et al. 2012, 2013). Experimental evidence showed that MPA induces Nm23 expression levels at higher dose in human breast carcinoma cell lines (Ouatas et al. 2003). Interestingly, high-dose-MPA exposure led to a reduction in anchorage-independent colonization which is further abrogated by Nm23-H1 antiserum transfection, suggesting an important role of MPA role for elevating Nm23 levels (Palmieri et al. 2005). Silencing of Nm23-H1 also has consequences for several biological

activities, including interruption of E-cadherin-mediated cell-to-cell adhesion, resulting in β -catenin nuclear translocation, T cell factor transactivation, deregulated cellular motility, and extracellular matrix invasion (Boissan et al. 2013, 2014). Recent studies by independent groups suggest that different viral oncoproteins have critical roles in regulating Nm23-H1 functions in the context of metastasis and cancer progression. One study demonstrated that the EBV essential latent antigen EBNA3C regulates cell migration by interacting with Nm23-H1 (Subramanian et al. 2001). Interestingly, this molecular association showed an important role for EBV in regulating the expression levels of different cellular proteins which are involved in cell migration and invasion (Kaul et al. 2009). Moreover, EBNA1 was also found to be associated with Nm23-H1 in lymphoblastoid cell lines (LCLs) leading to suppression of cell migration (Murakami et al. 2005). Other evidence suggested a role for KSHV in regulating Nm23-H1 which may be an important mechanism for KSHV induction, and targeting Nm23-H1 could be a possible therapeutic approach for the treatment of KS (Qin et al. 2011). Moreover, another report demonstrated the interaction of HPV-16 E7 with Nm23-H1 and Nm23-H2 in the human keratinocyte HaCaT cell line (Mileo et al. 2006). Interestingly, the expression level of the E7 oncoprotein in HaCaT cell line was observed to induce modified keratinocyte proliferation as well as differentiation patterns. This resulted in down-modulation and functional inactivation of Nm23-H1 (Mileo et al. 2006). Therefore, different viral antigens are important in deregulation of metastasis suppressor genes and are potentially useful for targeted therapeutic strategies against tumor virus-associated human malignancies.

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Fig. 1.

Role of Nm23-H1 in regulation of diverse cellular signaling activities. Nm23-H1 has histidine protein kinase activity toward its substrates aldolase C, ATP-citrate lyase, and KSR, a scaffold protein in MAP kinase signaling. Altered expression levels of Nm23-H1 resulted in degradation of KSR and low-level activation of ERK (Steeg et al. 2008). Partial Nm23-H1 and Cdc42 localization occurs in the cytoplasm. Dbl-1 exchanges GDP with GTP on Cdc42. Cdc42-GTP can trigger signal transduction to regulate diverse cellular functions. Nm23-H1 inhibits the phosphorylation of Dbl-1 and Cdc42 GDP/GTP exchange (Murakami et al. 2008). Upon DNA damage response, the Nm23-H1/STRAP complex is dissociated through p53 signaling and maintained at a basal level. Consequently, the association between Nm23-H1 and p53, or STRAP and p53, results in removal of Mdm2 from the p53/Mdm2 complex (Jung et al. 2007). This molecular event stimulates p53-mediated apoptosis and cell cycle arrest. Granzyme A-mediated cleavage of Nm23-H1 inhibitor TAF-I β and HMG-2 and APE1 initiates Nm23-H1-induced DNA and caspase-independent apoptosis (Schultz-Norton et al. 2008)

Fig. 2.

Role of viral oncoproteins in deregulation of Nm23-H1-associated functions. Following virus infection, vital cellular processes are deregulated by viral antigens, which include EBV-encoded EBNA3C and EBNA1, KSHV-encoded LANA, and HPV-encoded E7. Nm23-H1 is targeted by EBNA3C, EBNA1, and LANA resulting in translocation from the cytoplasm to the nucleus. EBNA3C forms a stable complex in the nucleus with Nm23-H1 (Subramanian et al. 2001). EBNA3C upregulates several promoters with Nm23-H1 including Cox-2, MMP-9, and α V integrin. Moreover, these activities are associated with modulation of different cellular processes including metastasis, mitogenesis, angiogenesis,

mutagenesis, and apoptotic inhibition (Kaul et al. 2006). Molecular interaction with Necdin and Nm23-H1 modifies Nm23-H1-linked NDP kinase activities (Kaul et al. 2009). EBV EBNA3C has a critical role in rescuing Necdin-mediated down-regulation of VEGF promoter in association with Nm23-H1 (Kaul et al. 2009). Notably, HPV encoded E7 associates with Nm23-H1 to inhibit Granzyme A-induced apoptosis and may also be important for development of treatment strategies for HPV-associated cancers

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Table 1

Related biological functions of Nm23-H1

Functions	Descriptions	References
Nucleoside diphosphate kinase (NDPK) activity	Human Nm23-H1 gene-encoded A-subunit of NDPK can be activated as homohehexamers or heterohexamers with the B-subunit encoded by nm23-H2 gene.	Bosnar et al. (2004)
Histidine kinase activity	Nm23-H1 exhibits protein histidine kinase activity that mediates its anti-motility functions.	Wagner and Vu (1995)
3'-5' exonuclease activity	Recent report demonstrated 3'-5' exonuclease activity for NM23-H1, which suggested a preference for DNA substrates which contain overhanging 3'-termini.	Ma et al. (2004)
Metastasis	Nm23-H1 possesses potential anti-metastasis activity.	Kaetzel et al. (2009)
Cell motility	Binding of Nm23-H1 with gelsolin is involved in the remodeling of cellular actin filaments as well as regulation of movement and cellular morphology.	Sun et al. (1999)
Cell proliferation	Inhibition of Nm23-H1 reduced the rate of cell proliferation and arrested cells in the G0/G1 phase of the cell cycle.	Jin et al. (2009)
Differentiation	Expression of Nm23-H1 gene has important role in modulation of cellular responsiveness to the microenvironment and tissue differentiation.	Howlett et al. (1994)
Apoptosis	Recent report suggested that Nm23-H1 may have a role for regulating cell cycle and apoptosis in human B cells.	Choudhuri et al. (2010)
Cell adhesion	Nm23-H1 reduces cell adhesion and migration properties in hepatocarcinoma by modulating glycosylation of integrin beta1.	She et al. (2010)