REVIEW

Targeting Tumor Metastasis by Regulating Nm23 Gene Expression

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Abstract

The Nm23 gene is a metastatic suppressor identified in a melanoma cell line and expressed in different tumors where their levels of expression are associated with reduced or increased metastatic potential. Nm23 is one of the over 20 metastasis suppressor genes (MSGs) confirmed *in vivo*. It is highly conserved from yeast to human, implying a critical developmental function. Tumors with alteration of the p53 gene and reduced expression of the Nm23 gene are more prone to metastasis. Nm23-H1 has 3'-5' exonuclease activity. This review focuses on the role of Nm23 in cancer progression and also a potential novel target for cancer therapy.

Keywords: Nm23 gene - metastasis suppressor - tumor metastasis - MSGs - matrix metalloproteinase proteins

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Introduction

Tumor metastasis is a process in which tumor cells leave primary tumor site to colonize other sites of the body lead to death for cancer patients. Metastasizing cells must first disseminate from the primary tumor, invade the surrounding tissue, intravasate and extravasate the circulatory system, initiate angiogenesis and colonize distant sites while evading the immune system (Chin-Shiu et al., 2005). Each step must successfully give rise to a metastatic tumor. Tumor metastasis are accountable for the cause of 90% of human cancer death instead of primary tumor (Sporn, 1996). To prevent these deaths, it is essential for the better understanding of the process and mechanism of tumor invasion and metastasis to identify a molecular target for cancer therapy. Metastasis is mechanism by which millions of cells are released by tumor into blood vasculature which disseminate and eventually proliferate at a discontinuous secondary site. (Siclari et al., 2006). During tumor progression many genes gains or loss in its functions that leads cancer cells to acquire the prerequisites for metastasis such as altered cell adhesion, uncontrolled proliferation, increased in motility, invasion and anchorage independent growth.

Non metastatic gene 23 (Nm23) was first identified on the basis of its reduced expression on highly metastatic murine melanoma (K-1735 TK) cells in year 1988 (Steeg et al., 1988; Stafforf and Vaidya, 2008). Nm23 is a nucleoside diphosphate kinase (NDPK) regenerating adenosine 5'–triphosphate (ATP) level for intracellular '*housekeeping*' enzyme by covalently transferring γ-phosphate from a nucleoside triphosphate (NTP) such as guanosine 5'-phosphate (GTP) to a nucleotide diphosphate acceptor through transphosphorylase mechanism (Buxton and Yokdang, 2011).

The Nm23 gene was identified whose expression is reduced in highly metastatic rodent tumors relative to the poorly metastatic tumor cells (Steeg et al., 1988; Marino et al., 2012). Nm23 gene is located on chromosome 17q 21 and codes for an 18.5-kDa protein containing 166 amino acids with NDPK and protein-histidine kinase activities, as well as serine auto phosphorylation activity (De La Rosa et al., 1995; Wagner and Steeg, 1997). The transfection of Nm23 c DNA into various cancer cell lines results in the suppression of metastatic potential such as in motility, invasion or colonization indicating that Nm23 as a potential metastasis suppressor gene that could function on the invasion and migration steps of the metastatic pathway (Khan et al., 2001; Liu et al., 2002). There are about eight human Nm23 genes were characterized, of which the H1 gene is most closely correlated with the metastatic phenotype in human breast carcinoma, colorectal carcinoma, ovarian carcinoma and hepatocarcinoma (Fuhrman et al., 2000; Sies and Stahl, 2003). In human tissues, the two most abundantly expressed Nm23 genes are Nm23-H1 and Nm23-H2 or NME1 and NME2, respectively. These genes encode the A and B subunits respectively of NDPK. Nm23 family protein involves in multiple biological functions in cell adhesion, cell migration, cellular differentiation, microtubule polymerization, signal transduction pathway, histidine dependent phosphorylation, vascular invasion, endocytosis, tumor cell shape and in apoptosis (Kimura et al., 2000; Krishnan et al., 2001; Otsuki et al., 2001; Fournier et al., 2003; Gallagher et al., 2003; Narayanan and Ramaswami, 2003; Sirotkovic-Skerley et al., 2005; Jung

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and Seong, 2007). Scientific evidence suggests that Nm23 has a dual role in tumor progression: i) over expression in primary tumors at early stages, ii) the association between the loss of Nm23-H1 expression in later stages and tumor aggressiveness and metastatic potential. Nm23 protein is highly conserved from the bacteria to human and this protein contributes substantial role in metastatic process by reduced level of Nm23 protein expression in metastatic lymph node rival to their consonant primary tumor, intimate that metastatic tumor cells originate from and are mainly composed of cell with low Nm23 protein expression (Ishikawa et al., 2003; Li and Chen, 2012). Differential gene expression of Nm23 gene down regulates five highly metastatic cell lines (Marshall et al., 2010). Ectopic expression of Nm23 suppress metastasis without altering primary tumor growth provides an evidence that the expression of specific genes is reduced in tumor cells that have acquired the ability to form metastasis (Marshall et al., 2010). Medroxyprogesterone acetate (MPA) has been reported to elevate Nm23 gene expression at high dose in MDA-MB-231 and MDA-MB-435 human breast carcinoma cell lines. The Nm23 promoter reveals that 248 base pair region containing a cassette for transcription factor binding sites present in the mouse mammary tumor virus long terminal repeat regulated by glucocorticoid response element could be a potential target for up-regulation of Nm23 gene (Ouatas et al., 2003; Marshall et al., 2010). Nm23 and its family members have a wide mechanisms that attributes its activity such as histidine kinase activity, binding of other protein to regulate metastatic formation and altered gene expression down steam of Nm23. Nm23 have been probable target for gene therapy i.e. Intra peritoneal injection of adeno-associated virus (AAV) transferred Nm23 gene increases its expression in the orthotopically implanted ovarian cells leads to increased in the survival time of the experimental animals. The exogenous gene, expressed in more than 95% of the tumor cells in nude mice have shown 60% reduction in the number of animals developing liver metastasis (Marshall et al., 2010). Therefore it is essential to develop an effective method in targeting metastatic cascade and inhibition of tumor progression.

Nm23 gene family isoforms

Nm23 family protein consist of eight genes encoded for NDPK such as Nm23-H1, Nm23-H2, Nm23-H3, Nm23-H4, Nm23-H5, Nm23-H6, Nm23-H7, Nm23-H8 and Nm23-LV which is derived from Nm23-H1 (Ishikawa et al., 2003; Quatas et al., 2003; Valentijn et al., 2006 Marshall et al., 2010). Two murine Nm23-1 and Nm23-2 and two human Nm23-H1 and Nm23-H2 of Nm23 genes which are 90 % identical of 17 KDa proteins (De La Rosa et al., 1995). Nm23-H1 which is perceived based on its reduced RNA expression in a very high metastatic murine melanoma cell lines. Nm23-H1 gene is a versatile kinase mapped to 17q.21, a locus (Mathieu et al., 2005). Nm23-H1 homolog is expressed in nucleus and differential expression of Nm23-H1 will enhance metastatic potential (Steeg et al., 1988; Stafford et al., 2008). Nm23-H2 homolog express similarly to that of Nm23-H1 and both protein are extended in function but expressed differentially according to the tissue (Postel et al., 2009). Nm23-H4 homolog is apparent among the gene family and localized within the mitochondria which are associated with outer and inner mitochondrial membrane (Milon et al., 2000). Nm23-H5 homologs are expressed in testis with ciliated cells like trachea and biliary tract (Munier et al., 2003). In human spermatozoa the gene Nm23-H5 is confined near to flagella microtubules (Munier et al., 2003). The expression of Nm23-H5 is testis is contract to Nm23-H1 and Nm23-H4 expression is depends upon the tissue. Nm23-H6 isoform (house keeping gene) are mitochondrial enzymes involves in conserve intracellular levels of NTP at the expense of ATP (Venturelli et al., 2000; Roymans et al., 2001). In addition to Nm23 family a neoteric protein Nm23-LV are overall expressed and encodes a protein consisting of the major part of Nm23-H1 and Nm23-H2 amino acids (Valentijn et al., 2006).

Nm23 gene family structure

Nm23-H1 consists of 154 amino acids of which 135 are conserved to Nm23-H2 homology. The protein amino acids such as phenylalanine 40, histidine 69, glutamic acid 93 and glutamine 147 were found in Nm23-1 protein (McDermott et al., 2008). The Nm23-H2 proteins have four stranded anti-parallel β sheet covered by six α-helices (Webb et al., 1995). Nm23-H2 proteins are hexamer contains Cys 145 in the upper molecules and one dimer is located near to Cys 145 in the lower molecules of the neighboring dimer (Webb et al., 1995). These residues represent the amino acid alaline in awd (abnormal wing discs). The inter-molecular disulphide bond between Cys 109 and Cys 145 were cysteine residue of Nm23-H2. These disulphide bonds provide flexibility to c-terminal sequences when two residues are 33 parts in the structure (Backer et al., 1993). The presence of Cys 145 in a conserved location provokes the formation of disulphide bond in all vertebrates. Where Nm23-H5 is a hexamer containing 55 amino acid extensions at COOH terminal end.

Differential expression of Nm23 gene family and regulation

Nm23-H1 as mRNA species which acquire as 10 folds higher in cells with low metastatic activity than in their highly metastatic analogue describing their role in metastatic progression. The low Nm23-H1 protein and mRNA expression in the tumor specimens associate with poor clinical prognosis and highly metastatic potential (De La Rosa et al., 1995). Nm23-H1 gene is strongly compelled by cellular differentiation. Transfer of low endogenous Nm23-H1 expression in breast cancer cell line, directs the cells to normal morphology and normal pattern of growth. Nm23-H1 c DNA which is transfected into human phaeochromocytoma cells (PCC) and instates nerve growth factor (NGF) and down regulates Nm23-H1 to stimulate cellular proliferation. The transpose of Nm23 reduces *in vitro* motility and colony formation in

response to growth factor including transforming growth factor beta (TGF-β). Nm23 family protein also regulate a growth regulatory signals induced by transforming growth factor-Beta 1 (TGF-β1), NGF, platelets derived growth factor (PDGF) and insulin like growth factor (IGF-1) (Otero, 2000). The over expression of Nm23-H1 protein afford variety of cancer such as breast cancer, esophageal squamous cell carcinoma (ESCC), prostratic lesions, dysplastic prostatic epithelium, neuroblastoma, thyroid, renal cell carcinoma and gastric cancer (Sirotkovic-Skerlev et al., 2005; Filiz et al., 2010; Li et al., 2010). Over expression of Nm23-H1 in H7721 cells obstruct the expression of some glycosyltransferases, impaired glycosylation of β1 intergrin precursor and down regulated intergrin β1 expression on cell surface resulting in the reduction of cells interaction with fibronectin that abrogated intracellular signals mediating focal adhesion, cell migration and cytoskeleton formation (She et al., 2010). Reduced expression of Nm23-H1 promotes metastatic potential in gastric carcinoma to regional lymph node lead to increases in lymphatic metastasis and aberration of transcription regulation (Tomita et al., 2001). Nm23-H2 are less involved in metastasis suppression to than Nm23-H1 homolog (Hamby et al., 2000). The author reported that polyclonal transfectant by Nm23-H2 protein does not initiate metastasis suppressive phenotype to MDA–MB-435 cells, but also the metastatic monoclonal cell lines have high level of Nm23-H2 expression. It is also notify that the over expression of Nm23-H2 inhibits cell migration and colonization (Syed et al., 2005). Nm23-H2 protein also involves in ERK signaling pathway responsible for cellular proliferation and transmission of signals through Ras/Raf/ERK cascade (Roberts and Der, 2004; Yu et al., 2004; Adams et al., 2005). Nm23-H2 over expression suppresses ERK activation and blocks the activation of Raf-1, MEK and ELK-1 regulated by ERK pathway and consecutive proliferation. Nm23-H2 also regulate cellular proliferation through the blocking the activation of Ras-ERK signaling pathway which is necessary for proliferation (Lee et al., 2009). Nm23-H2 involves in Lbc mediated signaling pathway mechanism (Iwashita et al., 2004). Nm23-H2 gene mainstay with Lbc in cells and present GTP to Rho GEF Lbc. The Rho A pulls down the assay and led to 3TC stress fiber formation, in this manner Nm23-H2 negatively regulate Lbc mediated signaling pathway (Iwashita et al., 2004). Nm23-H2 also has the properties to bind with single stranded pyrimidine rich paranemic form of DNA. Nm23-H2 also binds to c-myc and NHE have a G4 motif that withhold c-myc expression and Nm23-H2 act as an activator of c-myc expression and there is decrease levels of Nm23-H2 leads to lower c-myc levels (Simonsson et al., 2000; Siddiqui-Jain et al., 2002). The decreased expression of Nm23-H2 are associated with metastatic potential and reduction in Nm23-H2 level in cancer cells reduced in c-myc expression that decreases apoptosis of the cancer cells enhancing metastasis (Zajac-Kaye, 2001; Thakur et al., 2009). Nm23-H5 involves in nucleotide metabolism of the germ cells which are expressed in testis close similar to Nm23-H1, H2, H3 and H4 depending upon the type of the tissue. A typical protein Nm23LV consist of the major parts of the Nm23-H1 and Nm23-H2 amino acids and they lacks exon 5 of the Nm23-H1 gene which encodes for one β sheet and one α helix, thus Nm23-LV have seven β sheet (Valentijn et al., 2006). Nm23-LV is the derivative gene from Nm23-H1 which has similar function like reduction of tumor and tumorigenesis. The function of Nm23-LV is further embedding by the increase in Nm23-LV in neuroblastoma tumor patients (Valentijn et al., 2006). However, despite several proposed biochemical functions of Nm23, there is still a lack of correlation between NDPK activity of Nm23 proteins and their supposed anti-metastatic biological function. Nevertheless, there are also other mechanisms by which the Nm23 protein could act; serine phosphorylation levels that have been shown to correlate with the suppression of metastatic potential, histidine-dependent protein phosphotransferase activity indicated as being functionally involved in the metastasis suppressive effect of Nm 23-H1, transfer of phosphate on specific residues as aspartates or glutamates on other protein which correlates with the suppression of cell motility and last transcriptional activity on c-myc promoter through which Nm23 activates *in vitro* transcription of NDPK catalytic activity independently (Wagner et al., 1997). Nm23-H2/ NDP kinase B has been recognized as an activator of c-myc transcription via interactions with the NHE III_1 region of the c-myc gene promoter (MacDonald et al., 1993; Desvignes et al., 2009).

Tumor metastasis and metastasis suppressor genes

Metastasis is defined as spreading of malignant tumor cells from a primary tumor site to secondary organ and followed by the colonization and growth of these disseminated tumor cells in the secondary organ. Metastasis is the most significant contributor to cancer related morbidity and mortality. The molecular and cellular mechanism underpinning the multiple stages of the metastasis cascade are quite complex (Steeg, 2006). While specific mechanisms at each site were not completely understood (Chambers et al., 2002; Gupta and Massague, 2006; Steeg, 2006; Townson and Chambers, 2006). Tumor cells must acquire a motile and invasive phenotype for metastasis, allowing these cells to leave the primary tumor site followed by the invasion of tumor cells through a stromal tissue border and marked by changes in the adhesive and proteolytic abilities of the malignant cells and later cells invading through vascular endothelium or lymphatics, escape into blood or lymph vessels, respectively. Malignant cells must escape damage due to sheer forces, immune surveillance, and apoptosis induced by lack of substratum or anoikis. Once at a distant site the malignant cells lodge in a capillary bed where they adhere to the vessel walls by either changes in binding protein expression or physical size constraints. The malignant cells extravasate through the lining of blood vessel endothelial cells and basement membrane into the secondary organ where they must adjust to the new microenvironment. In metastasis, these cells must be survived in the secondary organ as single cells and to proliferate in order to promote metastatic colonies

Table 1. Nm23 Gene Family and its Regulations

Non Metastasis Gene	Chro-mosome location	Major expression site	Functions	Suppressor activity	Refs
Nm23-H1	17q21.3	Cytosol , Nucleus	GTPase activating protein, Regulation of Rho family GTPase, Rac1 specific nucle-otide exchange factor,Tiam-l.	Cell cycle arrest apoptosis, Anti-metastasis in breast cancer	(Marshall et al., 2009; Otsuki et al., 2001)
Nm23-H2	17.q21.3	Cytoplasm	Transcription regulation,Cell signaling, ERK pathway, Interaction with intergrin cy-toplasmic domain associated protein -1alpha.	Suppressor of breast cancer metastasis	(Valentijn et al., 2006)
Nm23-H3	16q13	Cytoplasm, Mito-chondrial fraction	Correlated to metastatic progression.	Reduces cell motility	(Carinci et al., 2007; Negroni et al., 2000)
Nm23-H4	16q13.3	Mitochondria	Energy pathway, association with outer and inner mito-chondrial membrane.	Control of apoptosis	(Milon et al., 2000)
Nm23-H5	5q31	Testis germinal cells	Involves in spermiogenesis, phosphotransfer network in spermiogenesis.	Metastasis suppres-sor	(Milon et al., 2000; McDermott et al., 2008; Choi et al; 2009)
Nm23-H6	3p21	Heart placenta, Skeletal muscles	Phosphotransferase activity, Cell cycle progression, Regu-lation of growth.	Growth suppressor, Affects cytokinesis	(Tsuiki et al.,1999)
Nm23-H7	1q24.2	Smooth muscles, Motile axonemes like trachea, Lungs, Testis	ATP binding ,nucleotide binding , Purine metabolism, Pyrimidine metabolism, GTP biosynthetic -process, magnesium ion binding. mi-crotuble binding property.	Suppressed basal cAMP formation and metastatic	(Ikeda et al., 2010; Gene report. BioGPS)
Nm23-H8	7p14.1	Human sperm, Cardiomyocytes	Involves in basal camp pro-duction, GTP biosynthesis, Cell redox homeostasis, UTP biosynthesis, Human sperm axonemal organization.	Suppressor of me-tastasis	(Sadek, et al., 2001; Gene report. BioGPS)
Nm23-LV	17q21.3	Cytoplasm, Kidney	Tumorigenesis.	Cell cycle arrest apoptosis, Anti-metastasis in breast cancer	(Valentijn et al., 2006)

(Townson and Chambers, 2006). This entire process is inefficient, as only a small fraction of tumor cells enter circulation from the primary tumor site to form overt metastasis (Chambers et al., 2000).

From the past few decades, interest has grown in the new field of metastasis suppressor genes (MSGs). These genes are functionally defined by their ability to suppress *in-vivo* development of metastases without affecting the growth of the primary tumor. Since the identification of the first of these MSGs in 1988 the number of validated MSGs has increased to over 20s (Steeg et al., 1988; Steeg, 2003; Rinker-Schaeffer et al., 2006). The majority of these MSGs have been identified by their reduced expression in metastatic cancer cells compared to congenic, non-metastatic cells using a wide variety of methods including microarray expression profiling, and subtractive library hybridization. Cell culture based assays, such as soft agar colony formation, wound scratch, and chemotaxis assays, have been used to quantify metastasis suppressive function *in vitro*, but only measure particular aspects of the metastatic process.

They are two classes of gene products in relation to metastasis; Internal factors that act inside the cell in a regulatory pathway i.e. Nm23 and the external factors that act outside the cell to block dissect the metastasis pathway i.e. cathepsin-D (Mona et al., 2000). Nm23 gene plays an important role in molecular level for the displacement of the tumor cells. The author reveals that the correlation of Nm23 and cathepsin-D will have more aggressive tumor with advancement on stage and grade. The cathepsin–D enhances the involvement in invasion and metastasis in cancer prostate.

Nm23: metastasis suppressor gene

Nm23 were the first discovered metastasis suppressor gene. Two murine (Nm23-Z and Nm23-2) and two human (Nm23-HZ and Nm23-H2) genes are identified, encoding -17 kDa proteins which are 90 % identical (De La Rosa et al., 1995). In a screen for genes differentially expressed

between tumorigenic, metastatic murine melanoma cell lines and related tumorigenic non metastatic lines, Nm23 expression are reduced in a highly metastatic samples (Steeg et al., 1988). Highly metastatic murine K-1735 TK melanoma cells were transfected with the murine Nm23-Z cDNA and empty vector as control. The *in-vivo* experimental (tail vein injection) and spontaneous (subcutaneous injection) metastatic potential of the Nm23-Z and control transfected cells are determined. In both assays, the Nm23-Z transfectants produced SO-90% fewer metastases than did the control transfectants. Expression of Nm23-Z does not correlate with a consistent decrease in anchorage-dependent or independent growth rates although the Nm23-Z transfectants exhibited an altered response to the cytokine transforming growth factor-p (TGF-P) in soft agar colonization assays. Several studies have demonstrated that metastatic competent tumor cells are often stimulated by TGF-P in colonization assays, while non-metastatic tumor cells are unresponsive or even inhibited by this cytokine. In agreement with these studies, the control transfectants were stimulated by TGF-P in a dose-dependent manner, while Nm23-Z transfectants exhibits no significant response. Similar trends were identified in other model systems. Low Nm23-H1expression in human tumors often correlated with poor patient survival although it is not considered to be an independent prognostic factor (Wang et al., 2004; Branca et al., 2006). Importantly, the transfection of Nm23 into highly metastatic K-1735 melanoma cells reduced their *in vivo* metastatic ability by 52% to 96%, with no effect on the primary tumor size. There are difference in tumor cell proliferation was observed *in-vitro*, in agreement with the primary tumor size data. Similar trends were observed in transfection experiments with breast, colon, oral, hepatocellular and melanoma cell lines (Tagashira et al., 1998). Using AAV gene therapy vector, an Nm23-H1 construct was delivered into an ovarian carcinoma model of peritoneal metastasis. It was expressed by the tumor cells and significantly extended mouse survival (Li et al., 2006). The metastasis suppressive effects of Nm23-H1 were confirmed when Lacombe and colleagues developed an Nm23 knockout mouse. When induced to develop hepatocellular cancer, the rate of tumor formation in the liver were unchanged between the knockout and wild-type mice; however, the knockout mice developed 2-fold more pulmonary metastasis (Boissan et al., 2005). Although eight human Nm23 homologues have been identified, only H1and H2 has been extensively studied for metastasis-related properties (Lacombe et al., 2000). Human Nm23-HZ cDNA were transfected into B16Fl0 malignant murine melanoma cell lines. The transfected melanoma cells have greater significant reduction in invasive and metastatic potential *in-vivo*, thus corroborating published Nm23 transfection data (Parhar et al., 1995). Several lines of evidence suggest that Nm23 may participate in the normal development and differentiation process as presented in Table 1. The Drosophila *awd* gene which are 77% identical and 96% homologous in predicted amino acid sequence to Nm23. How Nm23-H1 inhibits metastasis and, in particular, metastatic colonization has been the subject of intense research, with multiple false steps. Its mechanism of metastasis suppression studied on three levels including an assessment of its intrinsic biochemical activities, protein - protein interaction, and alteration of downstream gene expression. DNA methylation has been reported to have an impact on breast cancer metastasis (Ziaei et al., 2012). Elevations of Nm23-H1 expression in human breast carcinoma cell line, MDA-MB-231 are associated with demethylation of a specific CpG island. Treatment of these cell lines with 5-Aza-CdR significantly reduces their *in-vitro* motility, an important process in tumor cell metastasis (Hartsough et al., 2001). Matrix metalloproteinase proteins (MMPs) are defined as a family of enzymes which degrades extracellular membrane proteins playing an important role in tumor invasion and metastasis. Evidence show that rat homolog of Nm23-H1 (Nm23- β) down regulates MMP-2 and inhibits metastasis (Kuppers, et al., 2005).

Nm23 as a major contributor in down regulation of metastasis and tumor progression. However NDPK A/ Nm23-H1 promotes metastasis on NB69-derived human neuroblastoma (Almgren et al., 2004). Buxton, (2010) recently proposed that secreted sNDPK-B regulates growth and development of metastases by stimulating angiogenesis and may facilitate intravasation, migration and extravasation early in the metastatic process. Estrogen and its receptors play an important role in the activation and expression of Nm23-H1 and down regulation of metastasis promoter genes such as WAVE3 (an actin-polymerization *gene*), Lysyl oxidase (LOX) and Merm1/Wbscr22 has been reported to inhibit invasion and metastasis of cancer (Lin et al., 2004; Sossey-Alaoui et al., 2007; Nakazawa et al., 2011; Siddikuzzaman et al., 2011). T-cell lymphoma invasion and metastasis 1 (Tiam1), an important protein binds with NDPK-A and inhibits Tiam1 activity specific for Rac1 and therefore interfere with Rac1-mediated actin polymerization and lamellipodia formation, which are essential for cell adhesion and migration (Otsuki et al., 2001; Raftopoulou, and Hall 2004). Tiam1 protein was highly expressed in the lung tumor tissue which is closely related to lung cancer development and metastasis (Wang and Wang, 2012).

Role of Nm23 in p53 gene regulation

Tumor with altered p53 gene and reduced in expression of Nm23 gene are more prone to metastasis. The aggressive counterpart with a higher expression of Nm23-H1 protein are with lower tumor grade this phenomena is supported by interaction between Nm23-H1 with p53 genes inducing apoptosis and cell cycle arrest (Jung et al., 2007). Nm23-H1 and its binding counterpart serine threonine kinase receptor associated protein (STRAP) that regulate p53 and for its activity. The Cys 145 of Nm23–H1 and Cys152 of STRAP were necessary for p53 gene is necessary to bind with binding partners Nm23-H1 and STRAP. The author reports that the activation of p53 gene is by removing of Mdm-2 complex by Nm23-H1 and STRAP. Nm23-H1 with STRAP gene helps in positive regulation of p53 function for apoptosis and cell cycle arrest. Interaction between Nm23-H1 with p53 genes may lead to apoptosis and cell cycle arrest (Jung

et al., 2007).

The metastasis suppressor Nm23-H1 possesses 3'-5' exonuclease activity

The 3'-5' exonuclease are critical for maintenance of genomic stability through DNA repair, replication and recombination (Zhang et al., 2011). The loss of Nm23-H1 expression and its cognate 3'-5' exonuclease activity may be conceive to promote genomic instability and malignant progression. Although there may be possibility that 3'-5' exonucleases activity can exert a metastasis suppressor relevant function which is exclude on DNA repair process. The 3'-5' exonucleases and NDPK activities of Nm23-H1 is required for metastasis suppressor function. Zhang and his co-workers reported that human melanoma cell lines, 1205LU which metastasizes to the lungs with high penetrance in rodent experimental model of metastasis is deficient in the expression of both Nm23-H1 and Nm23-H2 protein isofoms (Zhang et al., 2011). The wild type Nm23-H1 inhibits motility and invasion capacity and also all mutant animals exhibited normal motility and invasion suppressing activity indicating that none of the activities (NDPK, hisK, and 3'-5' exonuclease) involves to these phenotype in the 1205LU melanoma cell lines. Analysis of metastasis suppressor activity of the panel of Nm23-H1 variants using spontaneous metastasis assay in athymic mice provides a complete method to analyze metastatic potential of cells *in-vivo* (Kaetzel et al., 2009). This assay provides a link that both 3'-5' exonuclease and NDPK activity is related to metastasis suppressor activity of Nm23-H1.

Nm23 proteins interact with DNA and Nm23-H2 has been shown to bind and activate the nuclease-hypersensitive element (NHE) of the c-myc promoter. Therefore it suggested a molecular mechanism of oncogenesis and malignant progression. NM23-H2 also can cleave the NHE sequence *in vitro* when presented in either linear or supercoiled plasmid form. So it plays a role in modulating transcription via remodeling of regulatory elements that exhibit non-B-form, or paranemic, DNA conformations (Postel et al., 2000). Each of these interactions with the NHE was independent of NDPK activity, as they were retained with an NDPK-defective mutant form (H118F) of the protein. The DNA cleaving activity of Nm23-H2 was further shown to occur via a DNA glycosylase/lyase-like mechanism, a hallmark of base excision DNA repair enzymes. Both Nm23-H1 and Nm23-H2 repress transcription via interactions with paranemic elements in the promoter region of the platelet-derived growth factor-A (PDGF-A) gene (Ma et al., 2002; Kaetzel, 2003). Repression of this oncogenic and metastasis promoting growth factor is consistent with a potential anti-metastatic function of Nm23 proteins. Nm23-H1 and Nm23-H2 also cleaved the PDGF-A regulatory elements *in vitro*; Nm23-H1 appeared to excise nucleotides progressively from the 3' terminus of single-stranded oligodeoxynucleotides, whereas Nm23-H2 appeared to cleave internally, as observed previously with the c-myc NHE sequence. Interestingly, the DNA cleavage function of Nm23 is conserved from the primordial NDPK gene in *Escherichia coli*. A study has also shown that Nm23-H1 is the DNA-cleaving component of a latent protein complex (SET gene) that is activated during cytotoxic T lymphocyte-mediated apoptosis (Levit et al., 2002). Evidence shown that Nm23-H1 have the 3'-5' exonucleases activity and this 3'-5' exonucleases are associated generally with DNA proofreading with loss of expression and/or function often associated with mutator phenotypes, and increased potential for cancer progression.

Nm23: novel drug target

Nm23 predominate role in metastasis suppressor made an advance to inhibit metastasis that contributes a new innovative research and possible drug target (Fan et al., 2003; Marshall et al., 2010). The expression of Nm23-H1 gene can be used to determine response treatment following radiotherapy for nasopharyngeal carcinomas and in ovarian cancer (Kapoor, 2009). Transfer of Nm23-H1 gene through adeno virus to prevent metastasis can be a rapid emerging and an improved version in therapeutic tool (Li et al., 2006; Kapoor, 2009). Modulating in Nm23-H1 gene expression can be used to draw to meet metastasis in malignant tumor because tumor metastasis is the leading cause of death in cancer patients (Marshall et al., 2009). Nm23-H1 gene product also serve as a marker of lymph node metastasis in lung cancer when Nm23-H1 expression is forcibly restored the metastasis to the lungs, lymph node and other organ are significantly decreased therefore Nm23-H1 expression level can be used as molecular target for cancer therapy (Marshall et al., 2009). The potential interest of Nm23 as drug target for its interaction with p53 gene indeed Nm23-H1 is up regulated by p53 and Nm23-H1 interact with STRAP that disrupted by p53 signals releasing both p53 and Nm23-H1 to binds Mdm2 thus p53 can regulate apoptosis and cell cycle arrest (Marshall et al., 2009). Drug like Lycopene has anti-migration and anti-invasion properties to SK-Hep-1 cells this mechanism is associated with the induced expression of Nm23-H1 gene. Reduced Nm23 expression may increase cisplatin resistant by down regulation of Nm23-H1 expression that decreases the incidence of lymph node metastasis in patients with neck and head cancer (Lizuka et al., 2000). The involvement of growth factors with Nm23 is also quite a complex scenario. There is much cross talk between the growth factors and the receptors in signaling. The ability of Nm23 to regulate a diverse set of cellular processes has been linked to their ability to modulate signal transduction by a diverse set of growth factors such as TGF- β 1, PDGF, ILGF and NGF (Otero, 2000; Seong et al., 2007).

Metastatic cascade mostly is not complete in the majority of the patients (Marshall et al., 2010). This provides an avenue for opportunity for clinician to exploit by adopting molecular targets like Nm23 which can be an appropriate mechanism from preventing colonization and the growth of large metastasis. This ensures the possibilities to have high significant impact over the patient's survival. Targeting metastasis suppressor gene like Nm23 has no effect on the growth of primary tumor

but extremely inhibits the process of metastasis and reduces the formation of metastasis (Steeg et al., 2008). For the past decades around twenty three genes has been included in metastasis suppressor gene among them Nm23 may be one of the molecular target in effective impact in preventing large metastasis.

Therapeutic approach to restore the anti-metastasis functions of Nm23-H1 can be adopted by using different methods including Nm23-H1 promoter activation by MPA treatment, activation of downstream, gene target and gene therapy (Marshall et al., 2010; Marino et al., 2012). The evidence shows that MPA elevates Nm23 expression at high dose in human breast carcinoma cell lines (Ouatas et al., 2003). MPA was known to analysis of Nm23-H1 promoter which reveals, a 248bp region regulating the reporter activity which contains transcription factor binding sites. This cassette is regulated by glucocorticoid response element that provides effective Nm23 targeting for their up regulation. Exposure to high dose MPA led to decrease in anchorage-independent colonization which is abrogated when the cells are transfected with antiserum Nm23-H1, confirming MPA role in elevating Nm23 levels (Ouatas et al., 2003; Palmieri et al., 2005). It is unbelievable that agent targeting a cascade in metastasis process may be capable of shrinking the well established metastasis. Therefore these targets can be used during the early stage of metastasis prior to the diagnosis of lesions.

Gene therapy may be one among the effective treatment in preventing metastasis. Nm23 is a reasonable target to attempt gene therapy for its multidisciplinary role in preventing metastasis. The evidence reveals that high efficient gene transfer accomplished by two or three intra peritoneal injection of adeno associated virus (AAV) increases Nm23 expression that leads to reduction in the development of liver metastasis that increased 35-days of survival time in ovarian cancer animals (Li et al., 2006).

Deliver of IONP-PLL- a novel non viral vector for efficient gene delivery along with plasmid Nm23 in to target tissue reduces lung metastasis of B16F10 melanoma cell line injected animal (Li et al., 2009). This proves that using nano-vectors targeting tissues can be useful systemic gene therapy. Combination of therapy such as gene therapy, chemotherapy and cyclophosphamide has been reported to increase survival time and also with greater suppression of metastasis cascade (Li et al., 2009). Multifunctional role of metastasis suppressor gene Nm23 family provides advancement in the field of new approach on targeted therapy. Nm23 has interdisciplinary role in biology of cell therefore it is necessary to study the enzyme that could be useful in improving the health condition of the tumor patients.

Conclusion

Nm23 expression has a significant role in targeting tumor metastasis. Decreased expression of the Nm23 family of genes has been associated with breast carcinoma in several studies and high expression clones exhibited a significant reduction in metastatic potential *in-vivo* and in additional clonal Nm23-H1 helps to reduce the expression of TGF-β (Howlett et al., 2010). Loss of Nm23-H1 expression correlates with the degree of metastasis and with unfavorable clinical prognosis in several types of human carcinoma. Nm23-H1 silencing disrupts cell-cell adhesion mediated by E-cadherin, results in β-catenin nuclear translocation and T-cell factor / lymphoid-enhancing factor-1 transactivation. Nm23-H1 silencing promoted cellular scattering, motility, and extracellular matrix invasion by promoting invadopodia formation and up regulating several MMPs, including membrane type 1 MMP. In contrast, silencing the related Nm23-H2 gene was ineffective at promoting invasion (Boissan et al., 2010). Nm23-H1 suppresses hepatocarcinoma cell adhesion and migration on fibronectin by modulating glycosylation of integrin beta1. One of the initial events triggered by stimulation of β1 integrin is the association of its cytoplasmic domain with focal adhesion kinase (FAK), a cytosolic non-receptor tyrosine kinase, which leads to the tyrosine phosphorylation and activation of FAK. Phosphorylated FAK is involve in activation of many signal transduction molecules and affects several cellular biological behaviors (She et al., 2010). Over expression of Nm23-H1, specifically its nuclear translocation may be a powerful predictor of radiation resistance in head and neck squamous cell carcinoma (HNSCC) (Park et al., 2010). Understanding the Nm23 gene expression could develop an effective method for targeting metastatic cascade and inhibition of tumor progression that could be a novel potential therapeutic strategy for cancer.

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