Nm23/Nucleoside Diphosphate Kinase in Human Cancers

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Tumor metastasis is the leading cause of death in cancer patients. From a series of tumor cohort studies, low expression of Nm23/NDP kinase has been correlated with poor patient prognosis and survival, lymph node infiltration, and histopathological indicators of high metastatic potential in a number of cancer types, including mammary and ovarian carcinomas and melanoma. In other tumor types, no correlation has been established. Transfection of Nm23/NDP kinase cDNA into highly metastatic breast, melanoma, prostate and squamous cell carcinomas, and colon adenocarcinoma cells significantly reduced the metastatic competency of the cells *in vivo*. In culture, cell motility, invasion, and colonization were inhibited, whereas tumorigenicity and cellular proliferation were not affected, indicating that Nm23/NDP kinase acts as a metastasis suppressor.

KEY WORDS: NDP kinase; Nm23; loss of heterozygosity; metastasis suppressor; NME genes.

Tumor metastasis is a complex network of cellular events leading to the spread of cancerous cells from the site of origin to distant sites where they establish secondary lesions. In order for the cells within the primary tumor to move beyond its borders into the surrounding tissue, alterations in cell–matrix attachment and cell–cell communication must ensue. This occurs by the cells overproducing or overactivating proteases to degrade extracellular matrix components, by changing the presence of adhesion molecules on the cell surface to reduce cell–cell attachments, by the misregulation of growth factors, and by the alteration in genes that usually keep the cell immobile. An accumulation of these changes result in cells that can leave the primary tumor site and invade the surrounding tissue and circulatory or lymphatic system. The cells are then passed through the body to distant organs where they extravasate and recolonize. In order to obtain the nutrients needed for survival, the cells secrete molecules that stimulate angiogenesis; after which, they colonize into secondary tumors.

With the complexity of the metastatic process, there are many potential levels of regulation. One such level is gene expression, in which the expression of certain genes are either "turned on" or "turned off," resulting in miscommunication within the cell. In a differential hybridization experiment comparing a group of highly metastatic murine melanoma cell lines with their related low metastatic counterparts, *Nm23/NDP kinase* was identified as a gene that was quantitatively underexpressed in metastatic cells at the RNA and protein levels. Since the initial isolation, the Nm23/NDP kinase family has grown to contain eight homologs (Lacombe *et al.*, 2000); however, only Nm23-H1 (Nm23/NDP kinase-A) and Nm23-H2 (Nm23/NDP kinase-B) have been extensively studied in human cancers and will, therefore, be the focus of this chapter (note: when the isoform is not specified, Nm23/NDP kinase refers to either Nm23/NDP kinase-A or to Nm23/NDPkinase-A and -B).

EXPRESSION PATTERNS IN TUMOR COHORTS

As the *Nm23/NDP kinase-A* gene was discovered in a murine melanoma metastasis model system, the

correlation of its expression with tumor metastatic potential in actual human cancers was a subject of great interest. Expression of Nm23/NDP kinase-A and -B at the protein level by immunohistochemistry or at the RNA level by *in situ* hybridizations, Northern blots, or RT-PCR has been extensively reported. This section will not be a comprehensive review of all the cohort studies, but it will provide key information about the significance of the expression patterns of Nm23/NDP kinases in human cancers.

Breast carcinoma and melanoma are the two tumor types most studied in this context. In both instances, a majority of reports cited an inverse relationship of Nm23/NDP kinase-A protein and/or RNA expression with metastatically aggressive disease. The latter can be defined many ways, by patient survival (overall, disease free, metastasis free), lymph nodal status (metastasis of primary tumor cells to the draining lymph nodes), or by primary tumor histopathological characteristics (nuclear or differentiation grade, expression of particular hormone receptors, etc.).

The breast cancer Nm23/NDP kinase cohort studies that we could identify by a database search are summarized in Table I as an example of the trends observed. Overall, 18 studies found a statistically significant correlation between reduced Nm23/NDP kinase-A expression and one of the aspects of metastatic aggressiveness mentioned above. In cases where multiple aspects of aggressiveness were examined and different associations were found [(for instance, no significant difference in disease-free survival (DFS), but a significant difference in nodal status), the nonsignificant (NS) parameters are also listed. Two general conclusions are evident: (1) The majority of studies confirm a trend between reduced Nm23/NDP kinase-A expression and some aspect of metastatic aggressiveness; and (2) Nm23/NDP kinase-A expression does not represent an independent prognostic factor in most studies reported. Independent prognostic factors may be capable of predicting the natural history of a disease statistically independent of other known indices (such as, nodal status and tumor size) and are identified by a multivariate analysis. These studies require a large number of tumors, long clinical follow-up, and accurate methodologies. It is notable that Heimann *et al.* (1998) reported that reduced Nm23/NDP kinase-A represented an independent prognostic factor in node-negative breast cancer (163 tumor cohort, 14 year follow-up), which is a subset of breast cancer patients needing better prognostic information. The only other node negative cohorts listed on Table I contained <50

tumors and were inadequate for multivariate analysis, so confirmation of this possibility is still awaited.

A number of factors are potentially significant when analyzing the data listed in Table I, including the size of the cohort, as previously mentioned. A second important variable is antibody specificity. Years ago, questions were raised as to the specificity of the anti-Nm23/NDP kinase antibodies with regard to recognition of higher molecular weight (non-Nm23/NDP kinase) bands on western blots. It is also noteworthy that, to date, eight Nm23/NDP kinase family members have been identified and it is not known which antibodies cross react to which subsets of this family. One trend is suggested herein: The antibody to NDPKA, an erythrocyte produced Nm23/NDP kinase preparation, produced no significant associations to patient clinical course (survival) in three of three studies, in contrast to many other antibodies cited. This antibody should not be used further in prognostic studies and investigation into its recognition pattern may permit the development of hypotheses concerning the biologically relevant Nm23/NDP kinase proteins. Another variable of interest is the manner in which "high-" and "low"-expressing tumors were discriminated. Most systems argued, on a biological basis, that an area of low-staining cells may designate a metastatically competent population, which could go on to kill the host. Therefore, in many studies, tumors with focal or diffuse low-staining areas were assigned to the "low"-expression category. When one eliminates studies with few tumors (< 50) and those IHC studies using the anti-NDPKA antibody, there are actually 13 studies which indicate a significant correlation of reduced Nm23/NDP kinase expression to either poor clinical course or nodal metastases (the two strongest predictors of metastatically aggressive disease), as opposed to two studies which do not—a convincing trend.

In melanoma, those patients that developed metastases during the first 2 years after diagnosis had significantly lower levels of primary tumor expression, compared to patients with less aggressive disease (Florenes *et al.*, 1992). Analogous correlation between low Nm23/NDP kinase-A expression and greater incidence of metastases and worse disease-free survival were reported by all other investigators (Betke *et al.*, 1998; Bodey *et al.*, 1997; Caligo *et al.*, 1994; Greco *et al.*, 1997; McDermott *et al.*, 2000; Xerri *et al.*, 1994), except one (Easty *et al.*, 1996). Similar to breast tumors, metastatic lesions extracted from melanoma patients had lower Nm23/NDP kinase-A expression than the primary tumor.

Table I Trends in Nm23/NDP Kinase Expression in Human Breast Carcinoma Cohort Studies[a]

Reference	N	Associations	Methodology
Significant associations between reduced expression and increased aggressiveness			
Midulla (1999)	71	Grade <0.05	IHC
Yoshida (1998)	82	Nodes 0.001	IHC, 1M2162 mAB (+ vs. focal low)
Charpin (1998)	168	MFS 0.012, OS, NS	IHC, Nm23-H1mAB (CAD)
Heimann (1998)	163N-	DFS 0.008, multivariate 0.03 (+ vs. focal low)	IHC, Nm23-H1mAB
Yamaguchi (1998)	102	DFS <0.01, nodes <0.01, multivariate 0.032[b]	IHC, mAB H1-229 (%+ and int.)
Bertheau (1998)	112	Nodes 0.007, young grade 0.011	IHC, antipeptide (%+ and int.)
Mao (1998)	101	OS 0.05, nodes 0.05	IHC
Han (1997)	100	DFS 0.0433, nodes 0.0036, multivariate 0.051	IHC[c] (>10%+ vs. <10% +)
Duenas Gonzalez (1996)	33	Nodes 0.00001	IHC, Nm23-H1mAB (+ vs. focal low)
Toulas (1996)	59	OS 0.001, MFS 0.001, ER 0.001	Western, chicken AB
Noguchi (1994b)	144	AX nodes 0.035, IM nodes 0.0146	IHC, antipeptide (intensity)
Tokunaga (1993)	130	OS 0.014, nodes <0.01	mAB H1-229 (>40%+ vs. <40%+)
Barnes (1991)	39	OS 0.03	Antipeptide (+ vs. focal low)
Nakopoulou (1999)	191	Stage NS, PgR 0.001	IHC, NDPKA
Russo (1996)	76	Differentiation <0.02, DFS, NS	IHC, NDPKA (%+ and int.)
Caligo (1997)	128	Nodes 0.007,	RNA, Northern
Hennessy (1991)	71	OS 0.003, DFS 0.002, nodes 0.02	RNA, Northern
Bevilacqua (1989)	17	Nodes 0.05	RNA, *in Situ*
No Significant Associations			
Russell (1997)	40N-	DFS, NS	IHC, antipeptide (>40%+ vs. <40%+)
Kapranos (1996)	44N-	DFS, NS	IHC, mAB
Sawan (1994)	197	OS, NS Grade, NS	IHC, NDPKA (intensity)
Noguchi (1994a)	124	Nodes, NS	IHC, antipeptide (intensity)
Albertazzi (1998)	47	Nodes, NS	RNA
Goodall (1994)	153	Nodes, NS	RNA

[a] Abbreviations: *N*, number of tumors analyzed. Also noted are any special characteristics of the tumor cohort: Grade, tumor grade; nodes, lymph nodal status; OS, overall survival; DFS, disease-free survival; MFS, metastasis-free survival; Multivariate, Cox's proportional hazards model of survival; ER, estrogen receptor expression; PgR, progesterone receptor expression; NS, not significant; IHC, immunohisto-chemistry; CAD, computer-assisted diagnosis, 3% surface area + vs. −; %+ and int., percentage of positively staining cells and intensity of stain; + vs. focal low, diffuse positive staining versus areas of low staining; AX, axillary; IM, internal mammary; N-, lymph node negative.
[b] Conducted with Nm23-H1 and Sialyl Lewis X antigen.
[c] Designates anti-Nm23/NDPK antibody, which could be either anti-NDPKA or anti-Nm23-H1mAB 301 from Novocastro laboratories.

Other types of cancer in which the majority of the evidence suggests an association between low expression of Nm23/NDP kinase and tumor aggressiveness and patient mortality are ovarian (Mandai *et al.*, 1994; Qian *et al.*, 1997; Scambia *et al.*, 1996; Srivatsa *et al.*, 1996; Veil *et al.*, 1995), hepatocellular (Iizuka *et al.*, 1995; Nakayama *et al.*, 1992; Shimada *et al.*, 1998; Yamaguchi *et al.*, 1994), laryngeal (Gunduz *et al.*, 1997; Lee *et al.*, 1996), nasopharyngeal (Guo *et al.*, 1998), esophageal squamous cell (Iizuka *et al.*, 1999b), and oral squamous cell (Lo Muzio *et al.*, 1999; Otsuki *et al.*, 1997) carcinomas. Colon and gastric cancers have also been widely studied; however, there is still no consensus on the importance of Nm23/NDP kinase expression. In addition, either no correlation or a positive association between tumor aggressiveness and Nm23/NDP kinase expression has been reported in testicular, prostrate, thyroid, endometrial and renal cell carcinomas, myeloid leukemia, and pulmonary adenomas.

As stressed above, the expression loss of Nm23/NDP kinase is not a significant factor in all tumor types, which is not unexpected, since cancers are noted for their genetic instability and heterogeneity. Nevertheless, reduction in Nm23/NDP kinase-A expression has consistently been connected to aggressive, metastatic disease and poor patient prognosis in melanoma, breast, ovarian, and hepatocellular carcinomas.

MUTATIONS OF NDP KINASES

From all of the cohorts examined, only one tumor type has been found to convey overexpressed, mutated

Nm23/NDP kinase proteins. Elevated levels of Nm23/NDP kinase-A detected in neuroblastomas were ascertained to be a consequence of gene amplification. *Nm23/NDP kinase-B* gene amplification was not detected (Leone *et al.*, 1993b). Furthermore, Nm23/NDP kinase A overexpression was indicative of poor patient prognosis and disease-free survival (Leone *et al.*, 1993b). Upon single-strand conformation polymorphism analysis of the tumors, a subpopulation of *Nm23/NDP kinase-A and -B* cDNAs that contained mutations was disclosed in late-stage tumors. Nm23/NDP kinase-B, sequenced from a stage IV tumor, exhibited a leucine to valine mutation at amino acid 48 (Leone *et al.*, 1993b) and, in an independent study, a serine to glycine point mutation at amino acid 120 was detected in Nm23/NDP kinase-A (Chang *et al.*, 1994). Mutations of the Nm23/NDP kinase proteins were only detected in advanced neuroblastoma; they were not observed in early stage disease or with other types of tumors. In general, mutated Nm23/NDP kinase proteins are rare occurrences in tumors, whereas expression differences are very common.

NME GENES/LOSS OF HETEROZYGOSITY

The genes that encode Nm23/NDP kinase-A and Nm23/NDP kinase-B, *NME1* and *NME2*, respectively, have been colocalized to chromosome 17 position q21 (Backer *et al.*, 1993). This region, which also contains the early-onset familial breast–ovarian cancer (BRCA1) gene, is altered or deleted frequently in breast and many other cancers (Narod *et al.*, 1991; Spurr *et al.*, 1993). Consequently, it is possible that the decrease in Nm23/NDP kinase expression is the result of gene deletion.

Two polymorphism variants of *NME1* were identified by RFLP analysis of human chromosomal DNA from a normal population, indicating that there are two alleles of this gene (Yague *et al.*, 1991). In one study, allelic deletion of *NME1* was analyzed in 109 paired normal vs. cancer samples. Deletion of at least one of the *NME1* alleles was detected in 64% of human breast carcinomas, 42% of non-small cell lung carcinomas, 20% renal carcinomas, and 22% of invasive colon carcinomas. Tumors without detectable *NME1* allelic deletions were squamous cell carcinomas, large cell carcinomas, and other adenocarcinomas (Leone *et al.*, 1991b). Corroboration of *NME1* loss of heterozygosity (LOH) in breast carcinomas was reported in two other studies, in which 22–29% of the breast tumors exam-

ined exhibited allelic deletion of *NME1* (Futreal *et al.*, 1992; Watatani *et al.*, 1993).

Although LOH of *NME1* was correlated, in one report, to metastatic status in breast tumors (Watatani *et al.*, 1993), there was no association between LOH and poor prognosis among breast cancer survivors (Cropp *et al.*, 1994). Similarly, no correlation was observed between LOH of *NME1* in renal tumors and patient survival (Bosnar *et al.*, 1997). In contrast, colorectal carcinoma studies conflict. Lamb *et al.* (1996) noted no evidence to link *NME1* LOH in colorectal carcinoma with any clinical or pathological features or metastatic potential; whereas, a comparison of 30 primary and matched liver secondary lesions found concordant genomic alteration in 72% of the cases (Berney *et al.*, 2000), implying that there may be a correlation between LOH and prognosis. Regardless, LOH status has not been found to predict Nm23/NDP kinase-A protein levels (Bosnar *et al.*, 1997; Cropp *et al.*, 1994; Sauer *et al.*, 1998), which has been associated with poor patient survival and metastatic potential (see Table I). Thus, the inconsistent deletion of *NME1* that is observed is most likely a cause of concurrent deletion of other tumor-regulating genes within the 17.q21 region. Therefore, a mechanism other than LOH (i.e., RNA stability or transcriptional regulation) is responsible for controlling Nm23/NDP kinase-A protein expression. In an attempt to ascertain the disparity between low- and high-Nm23/NDP kinase-A expressing breast carcinoma cells, we have cloned the *NME1* promoter region and have begun to examine the machinery essential for transcription regulation.

FUNCTION OF NDP KINASE AS A METASTASIS SUPPRESSOR: TRANSFECTION STUDIES

In multiple tumor cohorts, low protein and mRNA expression of Nm23/NDP kinase within the tumor specimens correlated with poor patient prognosis and survival, lymph node infiltration, and other histopathological indicators of high metastatic potential. In addition, mutations of Nm23/NDP kinases were very infrequently observed. Taken together, the data propose that the amount of Nm23/NDP kinase is the lacking component within metastatic cells. Thus, to determine whether Nm23/NDP kinase fulfills a causal role (i.e., decreased expression induces metastasis), transfection experiments were performed; the results of these studies are summarized in Table II.

Table II. Comparison of *Nm23/NDP Kinase* Transfectants to Control Transfectants in Tumor Cell Lines[a]

Cell type	Cell line	NDPK isoform	Primary tumor size	Decrease in Metastases	*In Vitro* effects	Reference
Breast	Human MDA-MB-435	A	Same	50–90%	↓Colonization ↓motility ↑differentiation = proliferation	Leone (1993a)
	Human MDA-MB-435	A,B	Same	90–100%	↑Phospholipid metabolism ↓ pHi, ↑ pHe	Bhujwalla (1999)
	Human MDA-MB-231	A,B	Same	44–46%	↓Motility	Russell (1998)
	Rat MTLn3	B	↑13%	48%	N/A	Fukuda (1996)
Melanoma	Murine K-1735-TK	A	Same	52–96%	↓Colonization = proliferation	Leone (1991a)
	Murine B16-FE7	A,B	Same	83%	= Proliferation	Baba (1995)
	Murine B16-F10	A	N/A	93%	↓ICAM, ↓invasion	Parhar (1995)
	Murine MelJuSo	A	Same	40–80%	N/A	Miele (1997)
Colon	Rat colon 26	B	N/A	94%	= Proliferation	Tagashira (1998)
Prostate	Human DU145	A	N/A	N/A	= Proliferation ↓colonization ↓invasion	Lim (1998)
Oral squamous	Human LMF4	B	N/A	73–98%	↑Proliferation	Miyazaki (1999)

[a] Percentage decrease in the incidence of metastases formed in the lungs of mice from cells transfected with *nm23/NDP kinase* cDNA measured by a metastasis assay; NDPK, NDP kinase; N/A, data not available; ICAM, intercellular adhesion molecule-1, pHi, intracellular pH; pHe, extracellular pH.

Metastasis can be measured in immune-compromised mice either by subcutaneous or orthotopic transplantation of the cells to form a primary tumor, which subsequently metastasizes (spontaneous metastasis), or by injecting cells into the tail vein, by-passing the formation of a primary tumor (experimental metastasis). Lesions detected in the lungs and/or other organs are the metastases (reviewed in Welch, 1997). To date, 10 *in vivo* metastasis studies have been reported, 4 in breast carcinoma cell lines, 4 in melanoma cell lines, 1 in a colon carcinoma cell line, and 1 in an oral squamous carcinoma cell line (Table II). Transfection of either Nm23/NDP kinase-A or -B into these highly metastatic cells reduced the number of lesions found within the lung 40–100%. In 6 of the 10 studies, no difference in primary tumor size among Nm23/NDP kinase transfectants and vector-control transfectants was observed. Fukuda *et al.* (1996) noted a slight increase (13%) in Nm23/NDP kinase-transfected tumor size; however, this was insignificant to the outcome. The remainder of the studies used experimental metastasis assays and did not measure primary tumor size.

Whereas *in vivo* mouse studies measure overall tumorigenicity and metastatic competency of cells, the individual cellular events comprising the metastatic process can be tested *in vitro*. Several of these events, cell motility, colonization (measured in a soft agar assay), and invasion of cells through extracellular matrix, were significantly inhibited by Nm23/NDP kinase re-expression, without altering cellular proliferation. Hence, in all of studies, Nm23/NDP kinase overexpression decreased events leading to metastasis, subsequently reducing the metastatic competency of the cells without altering tumor proliferation. Nm23/NDP kinase is, therefore, not a tumor suppressor, but a metastasis suppressor gene, in that the formation of a tumor will occur regardless of Nm23/NDP kinase expression, but metastasis is significantly hindered with the presence of the gene product, suggesting a causal relationship.

How does Nm23/NDP kinase suppress metastasis? This question has yet to be completely answered; however, there have been several clues provided through the years. For example, normal mammary epithelial cells when grown in basement membrane components exhibit several aspects of the differentiation and development process, whereas breast carcinoma cells fail to recapitulate the process. When MDA-MB-435 human breast carcinoma cells were transfected with *nm23/NDP kinase-A* cDNA and reconstituted on extracellular matrix components, the overexpressing

Nm23/NDP kinase-A transfectants, unlike the vector-transfected controls, regained several normal differentiation functions, such as formation of ductal morphology, deposition of laminin and type IV collagen, expression of milk protein sialomucin, and inhibition of cellular growth (Howlett *et al.*, 1994). Similarly, transfection of *nm23/NDP kinase-A* cDNA into human pheochromocytoma cells induced growth arrest and initiated neurite differentiation (Gervasi *et al.*, 1996; Ishijima *et al.*, 1999). Thus, the normal function of Nm23/NDP kinase-A may be in the regulation of differentiation. The grade of differentiation has been correlated with aggressiveness in the histopathology of many tumor types and metastasis suppression may result from the acquisition of a differentiated phenotype.

Through what mechanism does Nm23/NDP kinase induce differentiation? Although it is very unclear in what signaling pathways Nm23/NDP kinases are involved, mutations within Nm23/NDP kinase-A at proline 96 and serine 120 inactivated the histidine-dependent phosphotransfer kinase activity of the protein and reversed the motility suppression observed with wild-type Nm23/NDP kinase upon transfection into breast carcinoma cells, thus linking a specific biochemical function of the protein with the metastasis suppressive capability (Freije *et al.*, 1997a; MacDonald *et al.*, 1996). Furthermore, primary tumors attained from MDA-MB-435 breast carcinoma cells transfected with *nm23/NDP kinase-A* or *-B* cDNA acquired an increase in both phospholipid metabolism (higher amounts of phosphodiester compounds relative to phosphomonoester compounds) and extracellular pH and a decrease in intracellular pH, when compared to control tumors (Table II) (Bhujwalla *et al.*, 1999). Although not proved, it is possible that the involvement of the phospholipid signaling and a microenvironmental pH-related mechanism may facilitate the Nm23/NDP kinase-dependent signaling pathway, through the phosphotransfer kinase activity, to mediate cell motility.

POTENTIAL IMPORTANCE OF Nm23/NDP KINASES TO CANCER THERAPY

In many instances, when patients are diagnosed with cancer, they also present with occult metastases. Primary tumors, which can be surgically resected, are not usually the cause of death for these patients; instead, metastatic lesions, whether by direct organ compromise or side effects from therapy, are the major contributors to patient mortality. It is, therefore, critical to define genes that contribute to or regulate the metastasis process in order to fully understand the disease and to develop effective treatments.

We know that reduced Nm23/NDP kinase expression is exhibited in a proportion of aggressive breast and ovarian carcinomas, melanoma, and some other cancers and that reintroduction of Nm23/NDP kinase to highly metastatic cells significantly reduced their metastatic proclivity. This occurred concurrently with reduction of cell motility, invasion, and colonization—all necessary events for establishing metastatic lesions. Thus, one could hypothesize that enhancing the expression of Nm23/NDP kinase in these cancer types, thereby suppressing many of the functions essential for successful completion of the metastatic series, would be of significant therapeutic benefit. We are, therefore, in the process of studying the promoter region of the *NME1* gene to determine what treatments would stimulate the endogenous up-regulation of the gene product.

Another therapeutic potential is to utilize Nm23/NDP kinase expression as a marker. For example, elevating Nm23/NDP kinase-A in human breast and esophageal squamous carcinoma cells was found to induce an increase in sensitivity to cisplatin treatment, a common alkylating chemotherapeutic agent, in culture as well as tumors grown in mice (Ferguson *et al.*, 1996; Iizuka *et al.*, 1999a). Moreover, Freije *et al.* (1997b) discovered a compound that differentially inhibited growth and motility of metastatic cells containing low Nm23/NDP kinase-A protein expression. Hence, it may be possible to translate Nm23/NDP kinase-A tumor levels into the decision process of choosing what therapeutic agents would be most beneficial for the patient.

In conclusion, there is still much to be learned about metastasis; however, the identification of the metastasis suppressor function of Nm23/NDP kinase has greatly impacted and advanced the cancer field, as well as paved the way for the discovery of other metastasis suppressor genes. Increasing Nm23/NDP kinase protein expression within tumors characteristic for low expression may be extremely helpful in halting or eradicating the progression of the disease. Nevertheless, further research focusing on defining the Nm23/NDP kinase signal transduction pathway involved in controlling the metastatic behavior of the cell is needed. In this way, further agents can be designed to "by-pass" the expression loss of Nm23/NDP kinase

by stimulating downstream events, providing a second level of potential therapy.

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