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Review

Metastasis suppressor genes

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Summary. Metastasis is a major cause of cancer mortality. Metastasis is a complex process that requires the regulation of both metastasis-promoting and metastasis suppressor genes. The discovery of metastasis suppressor genes contributes significantly to our understanding of metastasis mechanisms and provides prognostic markers and therapeutic targets in clinical cancer management. In this review, we summarize the methods that have been used to identify metastasis suppressors and the potential clinical impact of these genes.

Key words: Metastasis, Metastasis suppressor, RNA interference, Functional genomics, Next generation sequencing

Introduction

Metastasis is a process in which primary tumors disseminate to secondary organs. It is a complex, inefficient, but deadly process (Paget, 1889; Weiss, 1990; Welch et al., 2000; Chambers et al., 2000; Fidler, 2003; Parker and Sukumar, 2003; Gupta and Massague, 2006; Steeg, 2006; Hurst and Welch 2011; Valastyan and Weinberg, 2011). The completion of this process requires coordination of the activation of metastasis-promoting genetic programs and the inhibition of metastasis-suppressing programs in tumors, as well as tumor microenvironments that allows cancer cells to escape primary sites and grow in secondary organs (Leone et al., 1991; Lee et al., 1996; Seraj et al., 2000; Muller et al., 2001; Steeg, 2003; Eccles and Welch, 2007). Metastasis suppressors are molecules that inhibit metastasis formation without affecting primary tumor growth (Table 1). Metastasis suppressor genes affect many steps of the metastatic process. Their specific regulation in this process is critical for the understanding of tumor metastasis. Here we review the discovery, molecular mechanisms and potential clinical applications of metastasis suppressor genes.

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Discovery of metastasis suppressors

Metastasis suppressors were first identified using a microcell-mediated chromosome transfer (MMCT) approach. MMCT introduces chromosomes into intact recipient cells (Fournier and Ruddle, 1977). Human/mouse hybrid A9 donor cells carrying a single human chromosome containing potential metastasis suppressors were fused with recipient cells to generate microcell hybrids (Fournier and Ruddle, 1977). Spontaneous metastatic ability of these microcell hybrids was assayed in mouse models (Yoshida et al., 2000). Chromosomes 1, 6, 7, 8, 10, 11, 12, 16, and 17 were found to harbor metastasis suppressors (Ichikawa et al., 1991, 1992; Rinker-Schaeffer et al., 1994; You et al., 1995; Nihei et al., 1995; Miele et al., 1996, 1997 Phillips et al., 1996; Nihei et al., 1996; Chekmareva et al., 1997; Kuramochi et al., 1997; Matsuda et al., 1997; Luu et al., 1998; Mashimo et al., 1998; Seraj et al., 2000). Comparative genomic hybridization (cGH) and PCR were used to isolate the suppressor genes from these chromosomes. Breast cancer metastasis suppressor 1 (BRMS1) is one of the genes isolated by MMCT (Seraj et al., 2000). It was identified by the transfer of chromosome 11 into the highly metastatic breast cancer cell MDA-MB-435 using MMCT, and its subsequent differential display for the identification of this gene (Seraj et al., 2000). It has been shown that, in addition to breast cancer, BRMS1 suppresses metastasis in multiple tumor types including ovarian, bladder, melanoma and non-small cell lung carcinoma (Seraj et al., 2001; Shevde et al., 2002; Zhang et al., 2006; Smith et al., 2009). Yeast two-hybrid screen and co-immunoprecipitation demonstrated that BRMS1 interacts with multiple proteins including ARID4A and SUDS3, both of which are components of the SIN3 histone deacetylase chromatin remodeling complex

(Meehan et al., 2004; Hurst et al., 2008;). However, interactions of BRMS1 with ARID4A and SUDS3 are not required for metastasis-suppressing functions (Hurst et al., 2008; Silveira et al., 2009). The mechanisms of BRMS1 in metastasis are still under investigation. Clinically BRMS1 expression determined by immunohistochemistry (IHC) correlates with survival in breast cancer (Hicks et al., 2006; Flolova et al., 2009) and non-small cell lung carcinoma (Smith et al, 2009).

Differential gene expression was further developed to isolate metastasis suppressors. Before the genome-wide detection of gene expression became available, differential colony hybridization was developed to compare gene expression from two different cell populations with differential metastatic potentials. NM23 was the first metastasis suppressor isolated using this method (Steeg et al., 1988). NM23 has eight family members but only NM23-H1 and NM23-H2 suppress metastasis in multiple tumor types (Lacombe et al., 2000). NM23 expression can serve as a potential prognostic marker for survival in breast, ovarian, melanoma, gastric, hepatocellular and non-small cell carcinoma (Hartsough and Steeg, 2000; Mao et al., 2001; Niu et al., 2002; Katakura et al., 2002; Wang et al., 2004; Guan-Zhen et al., 2007). NM23 has multiple functions including exonuclease, genomic stability maintenance, NDP and histidine kinases (Biggs et al., 1990; Freije et al., 1997; Ma et al., 2004; Kaetzel et al., 2009). It affects multiple cellular pathways including the MAPK pathway and the cytoskeleton-organizing pathway, which contribute to its metastasis-suppressing functions (Hartsough et al., 2002).

The development of genome-wide gene expression technologies enables the determination of gene expression profiling in tumor cells and tissues. Gene expressions in cell lines with differential metastatic capabilities were compared (Kang et al., 2003; Minn et al., 2005; Bos et al., 2009). These genes include both protein-coding genes and noncoding RNAs. MicroRNAs (miRNAs) are single-stranded noncoding RNAs of 21-23 nucleotides (He and Hannon, 2004; Pillai et al., 2007). They are a novel class of gene regulators that function by binding the 3' untranslated regions of target messenger RNAs leading to either suppression of their translation or acceleration of their degradation (He and Hannon, 2004; Pillai et al., 2007). miRNA expression was compared in the MDA-MB-231 cell and its metastatic variant cell derived from MDA-MB-231. Six miRNAs were found to have lower expression in metastatic derivative cells than in parent cells. Among these six miRNAs, miR-335 and miR-126 suppress metastasis without affecting primary tumor growth (Tavazoie et al., 2008). miR-335 targets multiple pathways including SOX4, MERTK, PTPRN2 and TNC, which contributes to its metastasis-suppressing functions (Tavazoie et al., 2008). miR-335 expression is also correlated with metastasis-free survival in clinical breast cancer (Tavazoie et al., 2008).

A functional genomics approach provides another method to isolate metastasis suppressors. The development of RNA interference (RNAi) technology allows for gene suppression in mammalian cells. Forward genetic screens using a genome-wide RNAi library in a mouse model enables the identification of metastasis suppressors without a priori knowledge of their functions. A genome-wide short hairpin RNA (shRNA) library was introduced into non-metastatic mouse mammary tumor 168FARN cells that were transplanted into mouse mammary fat pads (Gumireddy et al., 2009). The development of lung metastases serves as a positive selection system. Cells with knockdown of a metastasis suppressor will disseminate from mammary fat pad to secondary organs such as lung. These metastatic cells in lung were isolated for the retrieval of shRNA by PCR. This functional genomics screen identified Krüppel-like factor 17 (KLF17) as a novel metastasis suppressor (Gumireddy et al., 2009). Ectopic expression of KLF17 in highly metastatic 4T1 cells suppresses its metastatic potential without affecting the growth of primary tumor in a mouse model (Gumireddy et al., 2009). KLF17 belongs to a family of transcription factors with 17 members (Gumireddy and Huang, 2010). The suppression of KLF17 promotes tumor cell epithelial-mesenchymal transition (EMT) which leads to cell invasion and metastasis (Gumireddy et al., 2009). Microarray analysis identified transcription factor Id1 as a direct target of KLF17 and mediates its functions in

Table 1. Metastasis suppressor genes.

Metastasis suppressor	Mechanism of action
AKAP12	PKA regulation
BRMS1	Transcription regulation
Caspase 8	Apoptosis
CDH1	Cell adhesion
CDH11	Cell adhesion
CD44	Hyaluronic acid receptor
CRSP3	Transcription regulation
DCC	Cell adhesion
DLC1	Rho-GTPase activation
DRG1	Angiogenesis
GAS1	Apoptosis
Gelsolin	Actin depolymerization
KAI1	Apoptosis
KISS1/KISS1R	Tumor dormancy maintenance
KLF17	Transcription regulation
LSD1	Chromotin remodeling
MAP2K4	MAPKK signaling
MKK4	MAPK signaling
MAK7	MAPK signaling
MicroRNA-335, 126	Suppression of SOX4, MERTK, PTPRN2, TNC
Nm23	MAPK signaling
PEBP1	Raf kinase inhibition
RhoGDI2	Rho signaling
RRM1	PTEN upregulation
TXNIP	Redox regulation

metastasis (Gumireddy et al., 2009). KLF17 expression is significantly downregulated and Id1 expression is upregulated in breast cancer metastasis, and the combination of KLF17 and Id1 expression may serve as a biomarker for breast cancer metastasis (Gumireddy et al., 2009).

A forward genetic screen in an *in vitro* model identified the metastasis suppressor GAS1 in melanoma (Gobeil et al., 2008). A genome-wide mouse RNAi library was introduced into poorly metastatic B16-F0 mouse melanoma cells, which were subsequently grown in 3D cell culture containing collagen and matrigel (Gobeil et al., 2008). Colony formation was used as a selection marker. shRNAs were retrieved from the cells that formed colonies in 3D culture. Knockdown of GAS1, one of the 22 candidate genes identified from the screen, promoted metastasis without affecting primary tumor growth (Gobeil et al., 2008). GAS1 suppresses metastasis by promoting apoptosis in disseminated cancer cells at secondary organs (Gobeil et al., 2008). GAS1 expression is downregulated in metastatic clinical samples (Gobeil et al., 2008).

The development of high-throughput sequencing and single nucleotide polymorphism (SNP) technologies allows for the detection of somatic mutations and genetic variations in clinical tumor samples at the genome-wide level. Next generation sequencing has identified potential candidate genes that play critical roles in metastasis including metastasis suppressor candidates. High-resolution SNP arrays were used to detect genetic variants in the primary tumor samples of colorectal cancer patients with or without liver metastasis (Ghadimi et al., 2006; Al-Mulla et al., 2006a; Sayagues et al., 2010). Primary tumors with known liver metastasis showed gain of chromosome 7p, 8q, 13q, and 20q, and loss of chromosome 1p, 8p, 9p, 14q, 17p and 22q. Genes that are located in the regions of chromosomal loss include MAP2K4, LLGL1, FBLN1, ELAC2, ALDH3A2, ALDH3A1, SHMT1, ARSA, WNT7B, TNFRSF13B, UPK3A, TYMP, RASD1, PEMT, and TOP3A. These candidate genes can potentially serve as metastasis suppressors and regulate the metastatic process in colorectal cancer.

Whole-genome sequencing was also used to identify mutations that are specific in clinical metastatic samples. Genome sequencing of a basal-like primary breast cancer, its matched brain metastases and normal tissues found two de novo mutations, SNED1 and FLNC, which are specifically for metastases (Ding et al., 2010). The functions of these two genes in the metastatic process have yet to be characterized. The development of low-cost sequencing technologies will enable larger numbers of tumor samples to be sequenced to validate these results and identify novel metastasis suppressors.

Clinical applications of metastasis suppressors

Although the impact of metastasis suppressors in current clinical practice is limited, metastasis suppressors can potentially serve as prognostic markers, therapeutic targets, and predictors for treatment response. NM23 expression has been determined in many tumor types. High NM23 expression has been found to be correlated with good prognosis in multiple tumor types (Bevilacqua et al., 1989; Hennessy et al., 1991; Royds et al., 1993; Tokunaga et al., 1993; Yamaguchi et al., 1994; Xerri et al., 1994; Toulas et al., 1996; Kawakubo et al., 1997; Charpin et al., 1998; Heimann et al., 1998; Müller et al., 1998; Ohta et al., 2000; Tas et al., 2002). For example, high NM23 expression is associated with overall survival and disease-free survival in breast cancer in several studies (Bevilacqua et al., 1989; Hennessy et al., 1991; Royds et al., 1993; Tokunaga et al., 1993; Toulas et al., 1996; Charpin et al., 1998; Heimann et al., 1998). The expression of KAI1, PEBP1 and RECK have also been shown to correlate with improved survival in multiple tumor types (Adachi et al., 1996, 1998; Huang et al., 1998; Sho et al., 1998; Muneyuki et al., 2001; Schindl et al., 2001; Hashida et al., 2003; Masui et al., 2003; Takeuchi et al., 2004; Takenaka et al., 2004; Al-Mulla et al., 2006b; Song et al., 2006; Wu et al., 2006; Chatterjee et al., 2008; Martinho et al., 2009; Xu et al., 2010; Zhang et al., 2012). High KAI1 or PEBP or RECK expression is associated with improved overall survival and disease-free survival in colorectal cancer (Hashida et al., 2003; Takeuchi et al., 2004; Al-Mulla et al., 2006b). The correlation between expression of other metastasis suppressors and good prognosis depends on tumor types. For example, high expression of CTGF is correlated with improved survival in colorectal cancer, non-small cell lung carcinoma and gallbladder cancer, but inversely correlated with survival in esophageal cancer and glioma (Xie et al., 2004; Lin et al., 2005; Chen et al., 2007; Alvarez et al., 2008; Zhou et al., 2009;). These clinical observations suggest that the functions of some metastasis suppressor genes are cancer cell-type and cell-context dependent.

Metastasis suppressors can also potentially be used to determine which patients are more likely to respond to a particular treatment. It has been shown that patients with NM23 -positive ovarian cancer respond better to cisplatin than patients with NM23-negative tumors (Scambia et al., 1996). Similar observations were also found in patients with esophageal squamous cell carcinoma (Iizuka et al., 1999). NM23 expression is correlated with increased survival after cisplatin treatment following surgery (Iizuka et al., 1999). These studies demonstrate that the expression of metastasis suppressors can potentially be used to select patients for particular treatments.

Unlike tumor suppressors, most metastasis suppressors are not mutated in the majority of clinical tumor samples, but rather the expressions are downregulated. This observation raises the possibility that the activation of these metastasis suppressors can

potentially block metastasis and improve survival. It has been shown that the promoter region of NM23 contains glucocorticoid response elements that can elevate NM23 expression (Ouatas et al., 2002). Treatment of human breast cancer cell lines with dexamethasone medroxyprogesterone acetate (MPA) dramatically increases NM23 expression (Ouatas et al., 2003). Treatment of MPA in a mouse model significantly decreases pulmonary metastases (Palmieri et al., 2005). Clinical trials are underway to determine whether MPA can be used to improve survival in patients (Steeg and Theodorescu, 2007; Marshall et al., 2009a,b).

Studies of another metastasis suppressor, RhoGDI2, found that RhoGDI2 suppresses endothelin 1 (ET1), a vasoconstrictor whose expression is correlated with higher clinical stage in bladder cancer (Titus et al., 2005). Administration of atrasentan, an ET1 antagonist currently used in a clinical trial, significantly reduced pulmonary metastasis in a mouse model (Titus et al., 2005). These results indicate that metastasis suppressors or their downstream targets can serve as potential therapeutic targets in cancer patients.

Perspectives

Considering the complexity of the metastatic process, it is likely that additional metastasis suppressor genes have yet to be isolated. Genome-wide study of gene expression, genetic variants, somatic mutations, alterations in epigenetic regulation in the tumor and genetic background of patients in clinical samples and laboratory models will identify novel metastasis suppressor candidate genes. Functional characterization of these candidate genes is required to differentiate *bona fide* metastatic suppressors without affecting primary tumor growth. Cross-talk between primary tumor, metastatic cells and tumor microenvironment is critical for the development of metastases. Systemic factors that mediate metastases formation and growth are also important in the metastatic process. These are under-studied areas. It is possible that some metastasis suppressors function in the communication between environment and metastatic cells.

The applications of metastasis suppressors in clinical patient care are currently limited. With the identification of novel metastasis suppressors, it is conceivable that they can serve as more robust prognostic markers and as predictors for the selection of patients who respond well to particular treatment. Some of the metastasis suppressors should be able to serve as therapeutic targets based on the effects they have on tumor cells and the pathways in which they are involved. It is also possible that better therapeutic effects will be achieved when drugs that target metastasis suppressors are used in combination with other treatments. The discovery of metastasis suppressor genes has transformed metastasis research and will continue to contribute to the transformation of metastasis into a curable or manageable disease.

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References

Adachi M., Taki T., Ieki Y., Huang C.L., Higashiyama M. and Miyake M. (1996). Correlation of KAI1/CD82 gene expression with good prognosis in patients with non-small cell lung cancer. Cancer Res. 56, 1751-1755.

Adachi M., Taki T., Konishi T., Huang C.I., Higashiyama M. and Miyake M. (1998). Novel staging protocol for non small cell lung cancers according to MRP 1/CD9 and KAI1/CD82 gene expression. J. Clin. Oncol. 16, 1397-1406.

Al-Mulla F., AlFadhili S., Al-Hakim A.H., Going J.J. and Bitar M.S. (2006a). Metastatic recurrence of early stage colorectal cancer is linked to loss of heterozygosity on chromosomes 4 and 14q. J. Clin. Pathol. 59, 624-630.

Al-Mulla F., Hagan S., Behbehani A.I., Bitar M.S., George S.S., Going J.J., Garcia J.J.C., Scott L., Fyfe N., Murray G.I. and Kolch W. (2006b). Raf kinase inhibitor protein expression in a survival analysis of colorectal cancer patients. J. Clin. Oncol. 24, 5672-5679.

Alvarez H., Corvalan A., Roa J.C., Argani P., Murillo F., Edwards J., Beaty R., Feldmann G., Hong S., Mullendore M., Roa I., Ibanez L., Pimentel F., Diaz A., Riggins G.J. and Maitra A. (2008). Serial analysis of gene expression identifies connective tissue growth factor expression as a prognostic biomarker in gallbladder cancer. Clin. Cancer Res. 14, 2631-2638.

Bevilacqua G., Sobel M.E., Liotta L.A. and Steeg P.S. (1989). Association of low nm23 RNA levels in human primary infiltrating ductal breast carcinomas with lymph node involvement and other histopathological indicators of high metastatic potential. Cancer Res. 49, 5185-5190.

Biggs J., Hersperger E., Steeg P.S., Liotta L.A. and Shearn A. (1990). A Drosophila gene that is homologous to a mammalian gene associated with tumor metastasis codes for a nucleoside diphosphate kinase. Cell 63, 933-940.

Bos P.D., Zhang X.H-F., Nadal C., Shu W., Gomis R.R., Nguyen D.X., Minn A.J., van de Vijver M.J., Gerald W.L., Foekens J.A. and Mssague J. (2009). Genes that mediate breast cancer metastasis to the brain. Nature 549, 1005-1009.

Chambers A.F., Naumov G.N., Vantyghem S.A. and Tuck A.B. (2000). Clinical implications of experimental studies on metastatic inefficiency. Breast Cancer Res. 2, 400-407.

Charpin C. Garcia S., Bonnier P., Martini F., Andrac L., Horschowski N., Lavaut M. and Allasia C. (1998). Prognostic significance of Nm23/NDPK expression in breast carcinoma, assessed on 10-year follow-up by automated and quantitative immunocytochemical assays. J. Pathol. 184, 401-407.

Chatterjee D., Sabo E., Tavares R. and Resnick M.B. (2008). Inverse association between Raf Kinase Inhibitory Protein and signal transducers and activators of transcription 3 expression in gastric adenocarcinoma patients: implications for clinical outcome. Clin. Cancer Res. 14, 2994-3001.

Chekmareva M.A., Hollowell C.M., Smith R.C., Davis E.M., LeBeau M.M. and Rinker-Schaeffer C.W. (1997). Localization of prostate cancer metastasis-suppressor activity on human chromosome 17. Prostate 33, 271-280.

Chen P., Li W., Wang Y., Zhao S., Li D., Feng L., Shi X., Koeffler H.P., Tong X. and Xie D. (2007). Expression of Cyr61, CTGF, and WISP 1 correlates with clinical features of lung cancer. PLoS One 2, e534.

Ding L., Ellis M J., Li S., Larson D.E., Chen K., Wallis J.W., Harris C.C., McLellan M.D., Fulton R.S., Fulton L.L., Abbott R.M., Hoog J., Dooling D.J., Koboldt D.C., Schmidt H., Kalicki J., Zhang Q., Chen L., Lin L., Wendl M.C., McMichael J.F., Magrini V.J., Cook L., McGrath S.D., Vickery T.L., Appelbaum E., DeSchryver K., Davies S., Guintoli T., Lin L., Crowder R., Tao Y., Snider J.E., Smith S.M., Dukes A.F., Sanderson G.E., Pohl C.S., Delehaunty K.D., Fronick C.C., Pape K.A., Reed J.S., Robinson J.S., Hodges J.S., Schierding W., Dees N.D., Shen D., Locke D.P., Wiechert M.E., Eldred J.M., Peck J.B., Oberkfell B.J., Lolofie J.T., Du F., Hawkins A.E., O'Laughlin M.D., Bernard K.E., Cunningham M., Elliott G., Mason M.D., Thompson Jr. D.M., Ivanovich J.L., Goodfellow P.J., Perou C.M., Weinstock G.M., Aft R., Watson M., Ley T.J., Wilson R.K. and Mardis E.R. (2010). Genome remodeling in a basal-like breast cancer metastasis and xenograft. Nature 464, 999-1005.

Eccles S.A. and Welch D.R. (2007). Metastasis: recent discoveries and novel treatment. Lancet 369, 1742-1757.

Fidler I.J. (2003). The pathogenesis of cancer metastasis: the "seed and soil" hypothesis revisited. Nature Rev. Cancer 3, 1-6.

Flolova N., Edmonds M.D., Bondenstine T.M., Seitz R., Johnson M.R., Feng R., Welch D.R. and Frost, A. R. (2009). A shift from nuclear to cytoplasmic Breast Cancer Metastasis Suppressor 1 expression is associated with highly proliferative estrogen receptor-negative breast cancer. Tumor Biol. 30, 148-159.

Fournier R.E. and Ruddle F.H. (1977). Microcell-mediated transfer of murine chromosomes into mouse, Chinese hamster, and human somatic cells. Proc. Nat. Acad. Sci. USA 74, 319-323.

Freije J.M.P., Blay P., MacDonald N.J., Manrow R.E. and Steeg P.S. (1997). Site-directed mutation of Nm23-H1. Mutations lacking motility suppressive capacity upon transfection are histidine-dependent protein phosphotransferase pathways *in vitro*. J. Biol. Chem. 272, 5525-5532.

Ghadimi B.M., Grade M., Monkemeyer C., Kulle B., Gaedcke J., Gunawan B., Langer C., Liersch T. and Becker H. (2006). Distinct chromosomal profiles in metastasizing and non-metastasizing colorectal carcinomas. Cell Oncol. 28, 273-281.

Gobeil S., Zhu X., Doillon C.J. and Green M.R. (2008). A genome-wide shRNA screen identified GAS1 as a novel metastasis suppressor gene. Genes Dev. 22, 2932-2940.

Guan-Zhen Y., Ying C., Can-Rong N., Guo-Dong W., Jian-Xin Q. and Jie-Jun W. (2007). Reduced protein expression of metastasis-related genes (nm23, KISS1, KAI1 and p53) in lymph node and liver metastasis of gastric cancer. Int. J. Exp. Pathol. 88, 175-183.

Gumireddy K. and Huang Q. (2010). Identification of metastasis genes by a functional genomics approach. Cell Cycle 9, 423.

Gumireddy K., Li A., Gimotty P.A., Klein-Szanto A.J., Showe L.C., Katsaros D., Coukos G., Zhang L. and Huang Q. (2009). KLF17 is a negative regulator of epithelial-mesenchymal transition and metastasis in breast cancer. Nature Cell Biol. 11, 1297-1304.

Gupta G.P. and Massague, J. (2006). Cancer Metastasis: Building a framework. Cell 127, 679-695.

Hartsough M.T. and Steeg P.S. (2000). Nm23/nucleoside diphosphate kinase in human cancers. J. Bioenerg. Biomem. 32, 301-308.

Hartsough M.T., Morrison D.K., Salerno M., Palmieri D., Ouatas T., Mair M., Patrick J. and Steeg P.S. (2002). Nm23-H1 metastasis suppressor phosphorylation of kinase suppressor of ras via a histidine protein kinase pathway. J. Biol. Chem. 277, 32389-32399.

Hashida H., Takabayashi A., Tokuhara T., Hattori N., Taki T., Hasegawa H., Satoh S., Kobayashi N., Yamaoka Y. and Miyake M. (2003). Clinical significance of transmembrane 4 superfamily in colon cancer. Br. J. Cancer 89, 158-167.

He L. and Hannon G.J. (2004). MicroRNAs: small RNAs with a big role in gene regulation. Nature Rev. Genet. 5, 522-531.

Heimann R., Ferguson D.J. and Hellman S. (1998). The relationship between nm23, angiogenesis, and the metastatic proclivity of node-negative breast cancer. Cancer Res. 58, 2766-2771.

Hennessy C., Henry J.A., May F.E.B., Westley B.R., Angus B. and Lennard T.W.J. (1991). Expression of the antimetastatic gene nm23 in human breast cancer: an association with good prognosis. J. Natl. Cancer Inst. 83, 281-285.

Hicks D.G., Yoder B.J., Short S., Tarr S., Prescott N., Crowe J.P., Dawson A.E., Budd, G.T., Sizemore S., Cicek M., Choueiri T.K., Tubbs R.R., Gaile D., Nowak N., Accavitti-Loper M.A., Frost A.R., Welch D.R. and Casey G. (2006). Loss of BRMS1 protein expression predicts reduced disease-free survival in hormone receptor negative and HER2 positive subsets of breast cancer. Clin. Cancer Res. 12, 6702-6708.

Huang C.I., Kohno N., Ogawa E., Adachi M., Taki T. and Miyake M. (1998). Correlation of reduction in MRP 1/CD9 and KAI1/CD82 expression with recurrences in breast cancer patients. Am. J. Pathol. 153, 973-983.

Hurst D.R. and Welch D.R. (2011). Metastasis suppressor genes: at the interface between the environment and tumor cell growth. Int. Rev. Cell Mol. Biol. 286, 107-180.

Hurst D.R., Xie Y., Vaidya K.S., Mehta A., Moore B.P., Accavitti-Loper M.A., Samant R.S., Saxena R., Silveira A.C. and Welch D.R. (2008). Alterations of BRMS1-ARID4A interaction modify gene expression but still suppress metastasis in human breast cancer cells. J. Biol. Chem. 283, 7438-7444.

Ichikawa T., Ichikawa Y. and Isaacs, J.T. (1991). Genetic factors and suppression of metastatic ability of prostate cancer. Cancer Res. 51, 3788-3792.

Ichikawa T., Ichikawa Y., Dong J., Hawkins A.L., Griffin C.A., Isaacs W.B., Oshimura M., Barrett J.C. and Isaacs J.T. (1992). Localization of metastasis suppressor gene(s) for prostatic cancer to the short arm of human chromosome 11. Cancer Res. 52, 3486-3490.

Iizuka N., Hirose K., Noma T., Hazama S., Tangoku A., Hayashi H., Abe T., Yamamoto K. and Oka M. (1999). The nm23 H1 gene as a predictor of sensitivity to chemotherapeutic agents in oesophageal squamous cell carcinoma. Br. J. Cancer 81, 469-475.

Kaetzel D.M., McCorkle J.R., Novak M., Yang M.M. and Jarrett S.G. (2009). Potential contributions of antimutator activity to the metastasis suppressor function of NM23-H1. Mol. Cell. Biochem. 329, 161-165.

Kang Y., Seigel P.M., Shu W., Drobnjak M., Kakonen S.M., Cordon-Cardo C., Guise T.A. and Massague J. (2003). A multigenic program mediating breast cancer metastasis to bone. Cancer Cell 3, 537-549.

Katakura H., Tanaka F., Oyanagi H., Miyahara R., Yanagihara K., Otake Y. and Wada H. (2002). Clinical significance of nm23 expression in resected pathologic-stage I, non-small cell lung cancer. Ann. Thor.

Surg. 73, 1060-1064.

Kawakubo Y., Sato Y., Koh T., Kono H. and Kameya T. (1997). Expression of nm23 protein in pulmonary adenocarcinomas: inverse correlation to tumor progression. Lung Cancer 17, 103-113.

Kuramochi H., Ichikawa T., Nihei N., Kawana Y., Suzuki H., Schalken J.A., Takeichi M., Nagafuchi A., Ito H. and Shimazaki J. (1997). Suppression of invasive ability of highly metastatic rat prostate cancer by introduction of human chromosome 8. Prostate 31, 14-20.

Lacombe M.L., Milon L., Munier A., Mehus J.G. and Lambeth D.O. (2000). The human Nm23/nucleoside diphosphate kinases. J. Bioenerg. Biomem. 32, 247-258.

Lee J., Miele M.E., Hicks D.J., Phillips K.K., Trent J.M., Weissman B.E. and Welch D. R. (1996). Kiss-1, a novel human malignant melanoma metastasis-suppressor gene. J. Natl. Cancer Inst. 88, 1731-1737.

Leone A., Flatow U., King C.R., Sandeen M.A., Margulies I.M.K., Liotta L.A. and Steeg P.S. (1991). Reduced tumor incidence, metastatic potential, and cytokine responsiveness of nm23-transfected melanoma cells. Cell 65, 25-35.

Lin B.R., Chang C.C., Che T.F., Chen S.T., Chen R.J., Yang C.Y., Jeng Y.M., Liang J.T., Lee P.H., Chang K.J., Chau Y.P. and Kuo M.L. (2005). Connective tissue growth factor inhibits metastasis and acts as an independent prognostic marker in colorectal cancer. Gastroenterology 128, 9-23.

Luu H.H., Zagaja G.P., Dubauskas Z., Chen S.L., Smith R.C., Watabe K., Ichikawa Y., Ichikawa T., Davis E.M., Le Beau M.M. and Rinker-Schaeffer C.W. (1998). Identification of a novel metastasis-suppressor region on human chromosome 12. Cancer Res. 58, 3561-3565.

Ma D.Q., McCorkle J.R. and Kaetzel D.M. (2004). The metastasis suppressor NM23-H1 possesses 3'-5' exonuclease activity. J. Biol. Chem. 279, 18073-18084.

Mao H., Liu H., Fu X., Fang Z., Abrams J. and Worsham M.J., (2001). Loss of nm23 expression predicts distal metastases and poorer survival for breast cancer. Int. J. Oncol. 18, 587-591.

Marshall J.C., Lee J.H. and Steeg P.S. (2009a). Clinical-translational strategies for the elevation of Nm23-H1 metastasis suppressor gene expression. Mol. Cell. Biochem. 329, 115-120.

Marshall J.C., Collins J., Marino N. and Steeg P.S. (2009b). The Nm23-H1 metastasis suppressor as a translational target. Eur. J. Cancer 46, 1278-1282.

Martinho O., Gouveia A., Silva P., Pimenta A., Reis R.M. and Lopes J.M. (2009). Loss of RKIP expression is associated with poor survival in GISTs. Virchows Arch. 455, 277-284.

Mashimo T., Watabe M., Cuthbert A.P., Newbold R.F., Rinker-Schaeffer C.W., Helfer E. and Watabe K. (1998). Human chromosome 16 suppresses metastasis but not tumorigenesis in rat prostatic tumor cells. Cancer Res. 58, 4572-4576.

Masui T., Doi R., Koshiba T., Fujimoto K., Tsuji S., Nakajima S., Koizumi M., Toyoda E., Tulachan S., Ito D., Kami K., Mori T., Wada M., Noda M. and Imamura, M. (2003). RECK expression in pancreatic cancer: its correlation with lower invasiveness and better prognosis. Clin. Cancer Res. 9, 1779-1784.

Matsuda T., Sasaki M., Kato H., Yamada H., Cohen M., Barrett J.C., Oshimura M. and Wake N. (1997). Human chromosome 7 carries a putative tumor suppressor gene(s) involved in choriocarcinoma. Oncogene 15, 2773-2781.

Meehan W.J., Samant R.S., Hopper J.E., Carrozza M.J., Shevde L.A., Workman J.L., Eckert K.A., Verderame M. F. and Welch, D.R. (2004). Breast cancer metastasis suppressor 1 (BRMS1) forms complexes with retinoblastoma-binding protein 1 (RBP1) and the mSin3 histone deacetylase complex and represses transcription. J. Biol. Chem. 279, 1562-1569.

Miele M.E., Robertson G., Lee J.H., Coleman A., McGary C.T., Fisher P.B., Lugo T.G. and Welch D.R. (1996). Metastasis suppressed, but tumorigenicity and local invasiveness unaffected, in the human melanoma cell line MelJuSo after introduction of human chromosome 1 or 6. Mol. Carcinog. 15, 284-299.

Miele M.E., De La Rosa A., Lee J.H., Hicks D.J., Dennis J.W., Steeg P.S. and Welch, D.R. (1997). Suppression of human melanoma metastasis following introduction of Chromosome 6 is independent of NME1 (Nm23). Clin. Exp. Metast. 15, 259-265.

Minn A.J., Gupta G.P., Siegel P.M., Bos P.D., Shu W., Giri D.D., Viale A., Olshen, A.B., Gerald W.L. and Massague J. (2005). Genes that mediate breast cancer metastasis to lung. Nature 436, 518-524.

Müller W., Schneiders A., Hommel G. and Gabbert H.E. (1998). Expression of nm23 in gastric carcinoma: association with tumor progression and poor prognosis. Cancer 83, 2481-2487.

Muller A., Homey B., Soto H., Ge N., Catron D., Buchanan M., McClanahan T., Murphy E., Yuan W., Wagner S.N., Barrera J.L., Mohar A., Verastegu E. and Zlotnik A. (2001). Involvement of chemokine receptors in breast cancer metastasis. Nature 410, 50-56.

Muneyuki T., Watanabe M., Yamanaka M., Shiraishi T. and Isaji S. (2001). KAI1/CD82 expression as a prognosic factor in sporadic colorectal cancer. Anticancer Res. 21, 3581-3587.

Nihei N., Ichikawa T., Kawana Y., Kuramochi H., Kugo H., Oshimura M., Killary A.M., Rinker-Schaeffer C.W., Barrett J.C., Isaacs J.T. and Shimazaki, J. (1995). Localization of metastasis suppressor gene(s) for rat prostate cancer to the long arm of human chromosome 10. Genes Chromosome Cancer 14, 112-119.

Nihei N., Ichikawa T., Kawana Y., Kuramochi H., Kugoh H., Oshimura M., Hayata I., Shimazaki J. and Ito H. (1996). Mapping of metastasis suppressor gene(s) for rat prostate cancer on the short arm of human chromosome 8 by irradiated microcell-mediated chromosome transfer. Genes Chromosome Cancer 17, 260-268.

Niu Y., Fu X., Lv A., Fan Y. and Wang Y. (2002). Potential markers predicting distant metastasis in axillary node-negative breast carcinoma. Int. J. Cancer 98, 754-760.

Ohta Y., Nozawa H., Tanaka Y., Oda M. and Watanabe Y. (2000). Increased vascular endothelial growth factor and vascular endothelial growth factor c and decreased nm23 expression associated with microdissemination in the lymph nodes in stage I non-small cell lung cancer. J. Thorac. Cardiovasc. Surg. 119, 804-813.

Ouatas T., Clare S.E., Hartsough M.T., De La Rosa A. and Steeg P.S. (2002). MMTV-associated transcription factor binding sites increase nm23 H1 metastasis suppressor gene expression in human breast carcinoma cell lines. Clin. Exp. Metast. 19, 35-42.

Ouatas T., Halverson D. and Steeg P.S. (2003). Dexamethasone and medroxyprogesterone acetate elevate Nm23 H1 metastasis suppressor gene expression in metastatic human breast carcinoma cells: new uses for old compounds. Clin. Cancer Res. 9, 3763-3772.

Paget S. (1889). The distribution of secondary growths in cancer of the breast. Lancet 1, 571-573.

Palmieri D., Halverson D.O., Ouatas T., Horak C.E., Salerno M., Johnson J., Figg W.D., Hillingshead M., Hursting S., Berrigan D., Steinberg S.M., Merino M.J. and Steeg P.S. (2005).

Medroxyprogesterone acetate elevation of Nm23 H1 metastasis suppressor expression in hormone receptor-negative breast cancer. J. Natl. Cancer Inst. 97, 632-642.

Parker B.S. and Sukumar S. (2003). Distant metastasis in breast cancer. Cancer Biol. Therapy 2, 14-16.

Phillips K.K., Welch D.R., Miele M.E., Lee J.H., Wei L.L. and Weissman B.E. (1996). Suppression of MDA-MB-435 breast carcinoma cell metastasis following the introduction of human chromosome 11. Cancer Res. 56, 1222-1227.

Pillai R.S., Bhattacharyya S.N. and Filipowicz W. (2007). Repression of protein synthesis by miRNAs: how many mechanisms? Trends Cell Biol. 17, 118-126.

Rinker-Schaeffer C.W., Hawkins A.L., Ru N., Dong J., Stoica G., Griffin C.A., Ichikawa T., Barrett J.C. and Isaacs J.T. (1994). Differential suppression of mammary and prostate cancer metastasis by human chromosomes 17 and 11. Cancer Res. 54, 6249-6256.

Royds J.A., Stephenson T.J., Rees R.C., Shorthouse A.J. and Silcocks P.B. (1993). Nm23 protein expression in ductal in situ and invasive human breast carcinoma. J. Natl. Cancer Inst. 85, 727-731.

Sayagues J.M., Fontanillo C., del Mar Abad M., Gonzales-Gonzales M., Saraquete M.E., del Carmon Chillon M., Garcia E., Bengoechea O., Fonseca E., Gonzales-Diaz M., De Las Rivas J., Munoz-Bellvis L. and Orfao A. (2010). Mapping of genetic abnormalities of primary tumours from metastatic CRC by high-resolution SNP arrays. PLoS One 5, e13752.

Scambia G., Ferrandina G., Marone M., Benedetti Panici P., Giannitelli C., Piantelli M., Leone A. and Mancuso S. (1996). nm23 in ovarian cancer: correlation with clinical outcome and other clinicopathologic and biochemical prognostic parameters. J. Clin. Oncol. 14, 334-342.

Schindl M., Birner P., Breitenecker G. and Oberhuber G. (2001). Downregulation of KAI1 metastasis suppressor protein is associated with a dismal prognosis in epithelial ovarian cancer. Gynecol. Oncol. 83, 244-248.

Seraj M.J., Samant R.S., Verderame M.F. and Welch D.R. (2000). Functional evidence for a novel human breast carcinoma metastasis suppressor, BRMS1, encoded at chromosome 11q13. Cancer Res. 60, 2764-2769.

Seraj M.J., Harding M.A., Gildea J.J., Welch D.R. and Theodorescu D. (2001). The relationaship between BRMS1 and RhoGDI2 gene expression to metastatic potential in lineage related human breast cancer cell lines. Clin. Exp. Metast. 18, 519-525.

Shevde L.A., Samant R.S., Goldberg S.F., Sikaneta T., Alessandrini A., Donahue H.J., Mauger D.T. and Welch D.R. (2002). Suppression of human melanoma metastasis by the metastasis suppressor gene, BRMS1. Exper. Cell Res. 273, 229-239.

Sho M., Adachi M., Taki T., Hashida H., Konishi T., Huang C.L., Ikeda N., Nakajima Y., Kanehiro H., Hisanaga M., Nakano H. and Miyake M. (1998). Transmembrane 4 superfamily as a prognostic factor in pancreatic cancer. Int. J. Cancer 79, 509-516.

Silveira A.C., Hurst D.R., Vaidya K.S., Ayer D.E. and Welch D.R. (2009). Over-expression of the BRMS1 family member SUDS3 does not suppress metastasis of human cancer cells. Cancer Lett. 276, 32-37.

Smith P.W., Liu Y., Siefert S.A., Moskaluk C.A., Petroni G.R. and Jones, D.R. (2009). Breast cancer metastasis suppressor 1 (BRMS1) suppresses metastasis and correlates with improved patient survival in non-small lung cancer. Cancer Lett. 276, 196-203.

Song S.Y., Son H.J., Nam E., Rhee J.C. and Park C. (2006). Expression of reversion inducing cysteine-rich protein with Kazal motifs (RECK) as a prognostic indicator in gastric cancer. Eur. J. Cancer 42, 101-108.

Steeg P.S. (2003). Metastasis suppressors alter the signal transduction of cancer cells. Nature Rev. Cancer 3, 55-63.

Steeg P.S. (2006). Tumor metastasis: mechanistic insights and clinical challenges. Nature Med. 12, 895-904.

Steeg P.S. and Theodorescu D. (2007). Metastasis: a therapeutic target for cancer. Nature Clin. Prac. Oncol. 5, 206-219.

Steeg P.S., Bevilacqua G., Kopper L., Thorgeirsson U.P., Talmadge J.E., Liotta L.A. and Sobel M.E. (1988). Evidence for a novel gene associated with low tumor metastatic potential. J. Natl. Cancer Inst. 80, 200-204.

Takenaka K., Ishikawa S., Kawano Y., Yanagihara K., Miyahara R., Otake Y., Morioka Y., Takahashi C., Noda M., Wada H. and Tanaka F. (2004). Expression of a novel matrix metalloproteinase regulator, RECK, and its clinical significance in resected non-small cell lung cancer. Eur. J. Cancer 40, 1617-1623.

Takeuchi T., Hisanaga M., Nagao M., Ikeda N., Fujii H., Koyama F., Mukogawa T., Matsumoto H., Kondo S., Takahashi C., Noda M. and Nakajima Y. (2004). The membrane-anchored matrix metallo-proteinase (MMP) regulator RECK in combination with MMP 9 serves as an informative prognostic indicator for colorectal cancer. Clin. Cancer Res. 10, 5572-5579.

Tas F., Tuzlali S., Aydiner A., Saip P., Salihoglu Y., Iplikci A. and Topuz, E. (2002). Prognostic role of nm23 gene expression in patients with ovarian cancer. Am. J. Clin. Oncol. 25, 164-167.

Tavazoie S.F., Alarcon C., Oskarsson T., Padua D., Wang Q., Bos P.D., Gerald W.L. and Massague J. (2008). Endogenous human microRNAs that suppress breast cancer metastasis. Nature 451, 147-152.

Titus B., Frierson Jr H.F., Conaway M., Ching K., Guise T., Chirgwin J., Hampton G. and Theodorescu, D. (2005). Endothelin axis is a target of the lung metastasis suppressor gene RhoGDI2. Cancer Res. 65, 7320-7327.

Tokunaga Y. Urano T., Furukawa K., Kondo H., Kanematsu T. and Shiku H. (1993). Reduced expression of nm23 H1, but not of nm23 H2, is concordant with the frequency of lymph-node metastasis of human breast cancer. Int. J. Cancer 55, 66-71.

Toulas C. Mihura J., de Balincourt C., Marques B., Marek E., Soula G., Roche H., and Farve G. (1996). Potential prognostic value in human breast cancer of cytosolic Nme1 protein detection using an original hen specific antibody. Br. J. Cancer 73, 630-635.

Valastyan S. and Weinberg R.A. (2011). Tumor metastasis: molecular insights and evolving paradigms. Cell 147, 275-292.

Wang Y.F., Chow K.C., Chang S.Y., Chiu J.H., Tai S.K., Li W.Y. and Wang L.S. (2004). Prognostic significance of nm23-HI expression in oral squamous cell carcinoma. Br. J. Cancer 90, 2186-2193.

Weiss L. (1990). Metastasis inefficiency. Adv. Cancer Res. 54, 159-211.

Welch D.R., Steeg P.S. and Rinker-Schaeffer C.W. (2000). Molecular biology of breast cancer metastasis: Genetic regulation of human breast carcinoma metastasis. Breast Cancer Res. 2, 408-416.

Wu Z., Conaway M., Gioeli D., Weber M. J. and Theodorescu D. (2006). Conditional expression of PTEN alters the androgen responsiveness of prostate cancer cells. Prostate 66, 1114-1123.

Xerri L., Grob J.J., Battyani Z., Gouvernet J., Hassoun J. and Bonerandi J.J. (1994). NM23 expression in metastasis of malignant melanoma is a predictive prognostic parameter correlated with survival. Br. J. Cancer 70, 1224-1228.

Xie D., Yin D., Wang H.J., Liu G.J., Elashoff R., Black K. and Koeffler H.P. (2004). Levels of expression of CYR61 and CTGF are prognostic for tumor progression and survival of individuals with gliomas. Clin. Cancer Res. 10, 2072-2081.

Xu Y.F. Yi Y., Qiu S.J., Gao Q., Li Y.W., Dai C.X., Cai M.Y., Ju M.J., Zhou J., Zhang B.H., and Fan J. (2010). PEBP1 downregulation is associated to poor prognosis in HCC related to hepatitis B infection. J. Hepatol. 53, 872-879.

Yamaguchi A., Urano T., Goi T., Takeuchi K., Niimoto S., Nakagawara G., Furukawa K. and Shiku H. (1994). Expression of human nm23 H1 and nm23 H2 proteins in hepatocellular carcinoma. Cancer 73, 2280-2284.

Yoshida B.A., Sokoloff M.M., Welch D.R. and Rinker-Schaeffer C.W. (2000). Metastasis-suppressor genes: a review and perspective on an emerging field. J. Natl. Cancer Inst. 92, 1717-1730.

You J., Miele M.E., Dong, C. and Welch D.R. (1995). Suppression of human melanoma metastasis by introduction of chromosome 6 may be partially due to inhibition of motility, but not to inhibition of invasion. Biochem. Biophy. Res. Comm. 208, 476-484.

Zhang S., Lin Q.D. and Di W. (2006). Suppression of human ovarian carcinoma metastasis by the metastasis-suppressor gene, BRMS1. Int. J. Gynecol. Cancer 16, 522-531.

Zhang Y., Cheng S., Zhang G., Ma W., Liu Y., Zhao R., Zhang Q. and Pang D. (2012). Low expression of RECK indicates a shorter survival for patients with invasive breast cancer. Cancer Sci. 103, 1084-1089.

Zhou Z., Cao W., Xie J., Lin J., Shen Z., Zhang Q., Shen J., Xu L. and Li E. (2009). Expression and prognostic significance of THBS1, Cyr61 and CTGF in esophageal squamous cell carcinoma. BMC Cancer 9, 291.

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