Differential nm23 gene expression at the fetal–maternal interface

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Summary The product of the nm23 gene has been proposed as a candidate tumour metastasis suppressor protein. A strong association has been observed between reduced expression of the nm23 gene and acquisition of metastatic behaviour in some tumour cells, including breast cancer and melanoma, but not in others, such as neuroblastoma and colon, cervical and thyroid cancers. During the early gestation period both human and murine trophoblast cells exhibit in vivo invasive properties. Such invasive properties, however, disappear in the late stage of gestation. In the present study, we examined the abundance of nm23 mRNA from various fetal–maternal interface tissues (uterus, decidua, placenta and embryo) during early (day 8), mid (day 14) and late (day 18) stages of gestation in CDI mice, in order to determine whether nm23 plays any anti-invasive and/or biological roles during gestation. nm23 was found to be expressed in all the tissues during the early and mid stages of gestation. The expression levels were, however, variable among different tissues and development stages. In the early stage, nm23 mRNA levels were the highest and similar among tissues from the uterus, decidua, placenta and embryo. In the mid stage, the mRNA levels were reduced significantly in the uterus, decidua and placenta, but not in the embryo. In the late stage, nm23 mRNA was further reduced to the extent that it could not be seen in the decidua, was barely seen in the uterus and was weakly present in the placenta. However, the mRNA level of the embryo in the late stage was still high and similar to the early stage. We also examined nm23 expression in trophoblast cells from normal human term placenta and a highly metastatic human choriocarcinoma cell line, JAR. nm23 expression was significantly higher in JAR than in normal placenta, indicating that nm23 does not appear to have an anti-metastatic function in this cell line. Several cytokines – interleukin 2 (IL-2), tumour necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ) – and prostaglandin E2 (PGE2) known to modulate tumour growth and metastasis were examined to determine whether they regulate nm23 expression in JAR in vitro. The B16F10 melanoma cell line was used as control. No effect was found in the JAR cell line, whereas TNF-α, IFN-γ and PGE2 down-regulated nm23 expression in the B16F10 cell line. We conclude that high nm23 expression may be associated with cell proliferation and not correlate with its anti-invasive activity in the early stage of gestation and in the JAR cell line. The constant high nm23 level in the fetal tissue throughout the gestation suggests that nm23 may play an important role in embryogenesis. Given the existence of a strong association between reduced nm23 expression and acquisition of metastatic behaviour in melanoma, the observation of down-regulation of nm23 expression by cytokines in B16F10 melanoma cells but not in JAR cells supports the notion that tissue-specific factors may be involved in the dissociation of nm23 expression from its antimetastatic activity in JAR cells.

It has recently been suggested that the protein product of nm23 gene plays an important role in tumour metastasis suppression (Rosengard et al., 1989; Steeg et al., 1989). The nm23 protein has substantial homology with the protein encoded by a Drosophila abnormal wing discs (awd) developmental gene and nucleoside diphosphate (NDP) kinase, which catalyses the phosphorylation of nucleoside diphosphate into nucleoside triphosphates (Biggs et al., 1989; Kimura et al., 1990; Wallet et al., 1990). The abundance of nm23 expression has been reported to be inversely correlated with metastatic potential in several rodent metastasis model systems: murine k-1735 melanomas (Steeg et al., 1988a; Leone et al., 1991), N-nitrosomethylurea-induced rat mammary tumours (Steeg et al., 1988a), mouse mammary tumour virus-induced tumours (Steeg et al., 1989) and ras–adenovirus 2 Ela-co-transfected rat embryo fibroblasts (Steeg et al., 1988b). The expression of human nm23 gene has also been found to be lower in human breast cancer specimens with high metastatic potential than in those with low metastatic potential (Bevilacqua et al., 1989; Barnes et al., 1991; Hennessy et al., 1991; Hirayama et al., 1991). Such a correlation, however, has not been observed in some other human tumours, such as colon cancer (Hait et al., 1991), neuroblastoma (Hait et al., 1991), some solid tumours including breast carcinoma (Lacombe et al., 1991) and thyroid carcinomas (Zou et al., 1993). The expression of the gene is increased equally in both high- and low-metastatic colon cancers. In neuroblastoma and thyroid carcinoma nm23 expression is positively associated with advanced disease stage (Hait et al., 1991; Zou et al., 1993).

Trophoblast cells of the blastocyst and of the normal first-trimester placenta share some phenotypic similarities with malignant cells, e.g. rapid proliferation and ability to invade neighbouring tissues, including basement membrane, during the process of implantation and placental development, but do not have the ability for unlimited growth or metastasis, as few trophoblast cells can be identified in the decidua, and trophoblasts rarely penetrate the maternal blood vessels (Yagel et al., 1988). In the present study, we examined the abundance of nm23 mRNA from various fetal–maternal interface tissues (uterus, decidua, placenta and embryo) during early (day 8), mid (day 14) and late (day 18) stages of gestation in CDI mice, in order to determine whether nm23 plays any anti-invasive and/or biological role during gestation. We also examined the nm23 mRNA levels in normal end-stage human placenta and in a highly metastatic human JAR choriocarcinoma cell line. Several cytokines and PGE2 known to modulate tumour growth and metastasis were examined to determine whether they regulate nm23 expression in JAR cells in vitro.

Materials and methods

Probes

A full-length human nm23-H1 cDNA probe was kindly donated by P.S. Steeg of the National Cancer Institute, Bethesda, MD, USA.

The oligonucleotide probe for 18S ribosomal RNA was synthesised and the sequence is as follows: 5'-GGTCAGCGC-TTCGTCGGCATGTAATAG-3'.
Mice

CD1 pregnant mice (Charles River, Margate, Kent, UK) were used for the study of nm23 expression during gestation. Six to eight mice were sacrificed on days 8, 14 and 18. The uterus, decidua, placenta and embryo were removed from the pregnant mice and immediately frozen in liquid nitrogen and stored at -70°C until processed.

Tumour cell lines

The tumour cell lines used in this study were B16F10, a highly metastatic murine melanoma cell line, kindly provided by I.J. Fidler, Anderson Cancer Center, Houston, TX, USA, and the human choriocarcinoma cell line JAR, obtained from American Type Culture Collection, Rockville, MD, USA. Both cell lines were maintained in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS), penicillin (100 U ml\(^{-1}\)), streptomycin (100 μg ml\(^{-1}\)) and fungizone (25 μg ml\(^{-1}\)) at 37°C in a humidified atmosphere containing 5% carbon dioxide.

RNA extraction and Northern hybridisation

Total RNA was extracted by the guanidinium thiocyanate–phenol–chloroform method (Chomczynski & Sacchi, 1987). A 20 μg aliquot of total RNA was fractionated on 1% agarose gel containing 2.2 M formaldehyde and blotted onto nylon membranes (Hybond-N, Amersham) by capillary transfer. The accuracy of RNA loading was monitored by ethidium bromide staining and/or by hybridisation to an oligo probe for 18S ribosomal RNA as previously described (Shi et al., 1991). The nm23-H1 cDNA probe was labelled with [α-\(^{32}\)P]dCTP to a specific activity of 10⁹ c.p.m. μg\(^{-1}\) using Pharmacia’s random primer labelling kit. Hybridisation was performed at 42°C for 18 h in 6 × SSPE, 10 mM EDTA, 5 × Denhardt’s solution, 0.5% SDS, 100 μg ml\(^{-1}\) denatured salmon testis DNA and 50% formamide. The membranes were then washed twice in 2 × SSPE at 65°C and exposed to Kodak XAR-5 film at -70°C with intensifying screens.

Following autoradiography, band intensities were quantitated by a Bio-Rad scanning densitometry and normalised by comparison with the 18S ribosomal band.

\[\text{Figure 1} \text{ Northern blot analysis of nm23 gene expression in murine fetal–maternal interface tissues. Total RNA was electrophoresed on agarose/formaldehyde gel and blotted onto a nylon membrane. Hybridisation was carried out with a full-length nm23-H1 cDNA probe (top) and an oligo probe for 18S ribosomal RNA to monitor RNA loading (bottom).}\]

RT–PCR and DNA sequencing analysis

A 5 μg aliquot of total RNA was reverse transcribed into cDNA in a 15 μl volume, using Pharmacia’s first-strand cDNA synthesis kit. Two PCR primers were synthesised, which flanked the coding region of nm23-H1 gene (5'-CAGCGCGGATTTAATCTAA-3’ and 5'-GGATGGTGAAGACGAAATGTGG-3’). A 3 μl volume of cDNA mix was used for PCR in a 50 μl volume. Samples were denatured at 94°C for 3 min and submitted to 30 cycles of amplification under the following conditions: 40 s denaturation at 94°C, 40 s annealing at 56°C and 40 s extension at 72°C. DNA sequencing was performed by the dyeoxy chain-termination method after cloning the PCR products into TA cloning vector (Invitrogen, CA, USA).

Regulation of nm23 expression by cytokines and PGE\(_2\):

B16F10 melanoma cells or JAR cells were cultured in 75 cm\(^2\) culture flasks (Costar, MA, USA) either alone or in the presence of IL-2 (1,000 units ml\(^{-1}\), Sigma, MO, USA), TNF-α (1,000 units ml\(^{-1}\), Sigma), IFN-γ (1,000 units ml\(^{-1}\), Sigma) and PGE\(_2\) (250 ng ml\(^{-1}\), Sigma) for 16 h at 37°C in a humidified incubator containing 5% carbon dioxide. Total RNA was then prepared from 1 × 10\(^7\) cells and probed for nm23 expression.

Results

nm23 expression in fetal–maternal interface tissues

nm23 mRNA level was examined in tissues from the uterus, decidua, placenta and embryo on days 8, 14 and 18 (Figure 1). The expression level of nm23 gene was quantitated by densitometry and compared with different stages of gestation (Figure 2). As shown in Figures 1 and 2, nm23 was expressed in all the tissues examined as early as day 8. The expression levels were, however, variable among different tissues and development stages. In the early stage (day 8), nm23 mRNA levels were high and similar among tissues from the uterus, decidua, placenta and embryo. In the mid stage (day 14), the mRNA levels were reduced significantly in the uterus, decidua and placenta, but not in the embryo. In the late stage, nm23 mRNA levels were further reduced to the extent that nm23 mRNA could not be seen in the decidua, was barely seen in the uterus and was weakly present in the placenta. The mRNA level in the embryo at this stage, however, was still high and comparable to the early stage.
Therefore, nm23 expression was highest in the early stage as compared to the mid (day 14) and late (day 18) stages of gestation in tissues from the uterus, decidua, and placenta. The mRNA level in the embryo was constantly high and not reduced in the development of the embryo.

**nm23 expression in human choriocarcinoma cell line JAR**

During the early stage of gestation, the high levels of nm23 expression in the placenta prompted us to ask whether this was the result of its anti-metastatic protection against highly invasive trophoblast cells or related to the rapid cell proliferation. To this end, we compared nm23 expression in JAR cells, highly invasive malignant cells, with those in normal human placenta. As shown in Figure 3 the nm23 mRNA level is, indeed, increased significantly in JAR cells.

To determine if point mutations, deletions or rearrangements of the nm23-H1 gene caused the increased nm23 mRNA levels, we amplified the full-length nm23-H1 cDNA from the JAR cell line by reverse transcription–polymerase chain reaction (RT–PCR). The resulting 540 bp PCR cDNA product was cloned into TA vector and subsequently sequenced. Two separate rounds of cDNA synthesis, PCR amplification, cloning and complete cDNA sequencing were performed in order to rule out the possibility of enzymatic errors in reverse transcription and, more commonly, in PCR amplification with Taq polymerase. A total of five clones (three from the first RT–PCR and two from the second RT–PCR) were sequenced. The first RT–PCR examined showed a change in codon 38 from CTG (leucine) to CTT (leucine) in one of the three clones sequenced. The second RT–PCR examined was completely identical with the published cDNA sequences (Rosengard et al., 1989), indicating that the change is an RT–PCR artifact (data not shown). Thus, no mutation was found in the coding region of the nm23-H1 gene.

**Regulation of nm23 expression by cytokines and prostaglandin E2**

It has been shown that several cytokines can modulate nm23 antimetastatic activity in melanoma cells in vivo (Leone et al., 1991). It is not clear whether this is a direct or indirect effect on nm23. It is also not clear whether this modulation can be generalised to different types of tumours. In this regard, we analysed nm23 mRNA levels in JAR choriocarcinoma cells, which were cultured either alone or with IL-2, TNF-α, IFN-γ, IL-2 plus IFN-γ or PGE2; for 16 h. PGE2 (not cytokine) was included in the experiment because previous reports have shown that PGE2 can promote tumour metastasis (Garacci et al., 1987; Fulton et al., 1991; Young et al., 1991). B16F10 melanoma cells were also included in the experiments as a control. As shown in Figure 4a, IL-2 has no effect on nm23 expression in B16F10 melanoma cells. A 4-fold reduction of nm23 transcripts was, however, observed when the cells were cultured with TNF-α or PGE2. A 2-fold reduction was noted with either IFN-γ alone or IFN-γ plus IL-2 treatment. Surprisingly, the cytokines and PGE2 have no effect on nm23 expression in JAR cells (Figure 4b).

**Discussion**

The data presented herein demonstrate differential nm23 expression among fetal–maternal interface tissues during gestation, the highest level being found in early stage of gestation. The high level of nm23 expression in the fetal–maternal interface tissues (uterus, decidua, placenta) may be involved in the protection of maternal tissues from highly invasive trophoblast cells during the early stage of gestation. The progressive reduction in nm23 expression in uterus, decidua and placenta coincides with the progression of gestation and the reduction of invasiveness of trophoblast cells. This suggests that the reduced nm23 mRNA may be associated with ageing of these tissues and that the need for nm23 antimetastatic activity is reduced in late stage of gestation. However, as evidenced by the high level of nm23 mRNA in the human choriocarcinoma cell line JAR, high nm23 expression appears to be associated with rapid cell proliferation instead of its antimetastatic activity, thus contradicting the above hypothesis.

Tumours derived from JAR choriocarcinoma cells are histopathologically similar to what has been described for spontaneous choriocarcinomas of the uterus in women, in which large multinucleated cells (syncytiotrophoblasts) are common. The invasiveness of JAR cells is similar to or even higher than that of a highly metastatic murine melanoma line B16F10 (Yagel et al., 1988), in which we have recently found that increased nm23 expression in B16F10 cells is associated with reduced pulmonary metastases (Parhar et al., submitted). The mechanism resulting in the apparent dissociation of high nm23 expression in JAR cells with its antimetastatic function is not clear. Tissue-specific factors may be involved. A recent study by MacDonald et al. (1993) has demonstrated that autophosphorylation of nm23 protein at serine residues, not its NDF kinase activity, correlates with suppression of tumour metastatic potential. CAMP in vitro and forskolin in vivo can inhibit the serine phosphorylation of nm23, suggesting that this phosphorylation pathway is regulated in signal
transduction. In JAR choriocarcinoma cells, a defect may be present in this phosphorylation pathway which could prevent nm23 expressing its antimetastatic activity.

Association of high nm23 expression with rapid cell proliferation has been indicated in certain tumours such as neuroblastoma (Hailat et al., 1991), colon cancer (Haut et al., 1991) and thyroid tumour (Zou et al., 1993), and supported by a study showing that nm23-H1 expression is related to cell proliferation (Keim et al., 1992). It is known that the nm23 gene product has NDP kinase activity which provides intracellular pools of nucleoside triphosphates (excluding ATP) required for nucleic acid synthesis (Liotta & Steeg, 1990; Gilles et al., 1991). In many systems, NDP kinases have been found to be associated with GTP-binding proteins, including elongation factor (Walton & Gill, 1975; Ohtsuki & Yokoyama, 1987), microtubules (Nickerson & Wells, 1984) and p21 (Ohtsuki et al., 1986) or GSK (Kimura & Shimada, 1988; Otero, 1990), suggesting that they are involved in processes such as protein synthesis, tubulin polymerisation in the mitotic spindle and cytoskeleton and signal transduction by supplying GTP to GTP-binding proteins. A recent study has shown that the protein encoded by nm23-H2, one of two closely related human nm23 genes, may be a transcription factor that turns on the cellular myc gene, which is known to have cancer-causing potential (Postel et al., 1993). Thus, a high level of nm23 expression could possibly induce pleiotropic effects on cellular functions.

The constant high level of nm23 expression in the developing fetus during gestation is interesting. It suggests that nm23 may play an important role in embryogenesis. The study by Lakso et al. (1992) has provided evidence that nm23 protein accumulation is coincident with the functional differentiation of multiple epithelial tissues in the developing mouse fetus.

We have shown previously that TNF-α, IFN-γ and PGE₂ can not only down-regulate nm23 expression in B16F10 cell line in vitro, but also modulate nm23 antimetastatic activity in vivo (Parhar et al., submitted). In vivo treatment with TNF-α or IFN-γ or PGE₂ in experimental mice injected with B16F10 cells increased the pulmonary metastases and reduced the overall survival period as compared with those without any pretreatment (Parhar et al., submitted). The effect of cytokines on nm23 expression was, however, not observed in JAR cells in this study. Given that a strong association was found between reduced nm23 expression and acquisition of metastatic behaviour in melanoma (Steeg et al., 1988a; Leone et al., 1991) and that no such an association exists in malignant trophoblast cells, the observation of down-regulation of nm23 expression by some cytokines in B16F10 cells but not in JAR cells supports the notion that tissue-specific factors may be involved in the dissociation of nm23 expression from its antimetastatic activity in JAR cells. It may also suggest that nm23 is not directly involved in the tumour metastasis suppression, but rather is an intermediate product in the tumour metastasis suppression process, and its expression and function could be modulated by tissue-specific factors.

The human nm23-H2 gene has been discovered. It encodes a protein with a predicted Mr = 17,000 and is 88% identical to the nm23-H1 protein sequence (Stahl et al., 1991). The nm23-H1 probe we used does not efficiently distinguish between the two nm23 mRNAs. Northern blot hybridisation of nm23-H2-specific probe to breast tumours and cell lines shows that nm23-H2 expression was also reduced in high metastatic potential tumour cells but to a lesser extent than nm23-H1 (Stahl et al., 1991), indicating that the two genes are regulated independently. It is thus possible that the observed nm23 expression in this study could be one of several distinct forms of nm23 that are variably expressed and regulated in different cell types and thus play different biological roles.

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References

BARNESS, R., MASOOD, S., BARKER, E., ROSEGAND, A.M., COGGIN, D.L., CROWELL, T., KING, C.R., PORTER-JORDAN, K., WARGOZT, E.S., LIOTTA, L.A. & STEEG, P.S. (1989). Association of low nm23 RNA levels in human primary infiltrating ductal breast carcinomas correlates with reduced patients survival. Am. J. Pathol., 139, 245-250.

BELICACQUA, G., SOBEL, M.E., LIOTTA, L.A. & STEEG, P.S. (1989). A Drosophila gene that is homologous to a mammalian gene associated with tumor metastasis codes for a nucleoside diphosphokinase. Cell, 63, 933-940.

CHOMCZYNSKI, P. & SACCHI, N. (1987). Simple-step method of RNA isolation by using acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem., 162, 156-159.

FULTON, A.M., ZHANG, S.Z. & CHONG, Y.C. (1991). Role of the prostat glandulin E2 receptor in mammary tumor metastasis. Cancer Res., 51, 2047-2050.

GAR, N., PAOLETTI & SANTORO, M.G. (1987). Prostaglandins in Cancer Research. Springer: Berlin.

GILLES, A.M., PRESECAN, E., VONICA, A. & LASCU, I. (1991). Nucleoside diphosphate kinase from human erythrocytes. J. Biol. Chem., 266, 8784-8789.

HAILAT, N., MEINHARD, M., MELHIM, R.F., ZHU, X-X., ECKERSKORN, C., BRODEUR, G.M., REYNOLDS, C.P., SEEGER, R.C., LOTT-SPEICH, F., STRAHLER, J.R. & HANASH, S.M. (1991). High levels of p19 nm23 protein in neuroblastoma are associated with advanced stage disease and with nm23-gene amplification. J. Clin. Invest., 88, 341-345.

HAUT, M., STEEG, P.S., WILSON, J.K.V. & MARKOWITZ, S.D. (1991). Induction of nm23 gene expression in human colonic neoplasms and equal expression in colon tumors of high and low metastatic potential. J. Natl Cancer Inst., 83, 712-716.

HENNESSY, C., HENRY, J.A., MAY, F.E., WESTLEY, B.R., ANGUS, B. & LENNARD, T.W. (1991). Expression of the anti metastatic gene nm23 in human breast cancer: an association with good prognosis. J. Natl Cancer Inst., 83, 281-285.

HIGASHI, M., SUZUKI, Y., MORITA, T., KOSHIMA, Y., KINOMURA, N., SHIMADA, N., ESAMI, Y., KURASHIMA, C., UTSUYAMA, M. & HIROKAWA, K. (1991). Positive relationship between expression of anti-metastatic factor (nm23 gene product or nucleoside diphosphate kinase) and good prognosis in human breast cancer. J. Natl Cancer Inst., 83, 1394-1398.

KEIM, D., HAILAT, N., MELM, R.H., ZHU, X.X., LASCU, I., VERNON, M., STRAHLER, J. & HANASH, S.M. (1992). Proliferation-related expression of p19 nm23 nucleoside diphosphate kinase. J. Clin. Invest., 89, 919-924.

KIMURA, N. & SHIMADA, N. (1988). Membrane associated nucleoside diphosphate kinase from rat liver. J. Biol. Chem., 263, 4647-4653.

KIMURA, N., SHIMADA, N., NOMURA, K. & WATANABE, K. (1990). Isolation and characterization of a cDNA clone encoding rat nucleoside diphosphate kinase. J. Biol. Chem., 265, 15744-15749.

LACOMBE, M.-L., SASTRE-GARAU, X., LASCU, I., VONICA, A., WALLET, V., THIERY, J.P. & VERNON, M. (1991). Overexpression of nucleoside diphosphate kinase (nm23) in solid tumors. Eur. J. Cancer, 27, 1302-1307.

LAKSO, M., STEEG, P.S. & WESTPHAL, H. (1992). Embryonic expression of nm23 during mouse organogenesis. Cell Growth Different., 3, 873-879.

LEONE, A., FLATOW, U., KING, C.R., SANDEEN, M.A., MARGULIES, M.K., LIOTTA, L.A. & STEEG, P.S. (1991). Reduced tumor incidence, metastatic potential, and cytokine responsiveness of nm23 transfected melanoma cells. Cell, 65, 25-35.

LIOTTA, L.A. & STEEG, P.S. (1990). Clues to the function of Nm23 and Awd proteins in development, signal transduction and tumour metastasis provided by studies of Drosophila discoideum. J. Natl Cancer Inst., 82, 1170-1173.

MACDONALD, N.J., ROSA, A.D.L., BENEDICT, M.A., FREIJ, J.M.P., KRTUSCH, H. & STEEG, P.S. (1993). A serine phosphorylation of nm23, and not its nucleoside diphosphate kinase activity, correlates with suppression of tumor metastatic potential. J. Biol. Chem., 268, 25780-25789.

NICKERSON, J.A. & WELLS, W.W. (1984). The microtubule-associated nucleoside diphosphate kinase. J. Biol. Chem., 259, 5729-5732.

OHITSUKI, K. & YOKOYAMA, M. (1987). Direct activation of guanine nucleotide binding proteins through a high-energy phosphate-transfer by nucleoside diphosphate-kinase. Biochim. Biophys. Res. Commun., 148, 300-308.

OHITSUKI, K., IZUCHI, T. & YOKOYAMA, M. (1986). Characterization of nucleoside diphosphate kinase-associated guanine nucleotide-binding proteins from Hela S3 cells. Biochim. Biophys. Acta, 882, 322-330.

OTERO, A.D. (1990). Transphosphorylation and G protein activation. Biochem. Pharmacol., 39, 1399-1404.

PARHAR, R.S., SHI, Y., ZOU, M., FARID, N.R., ERNST, P. & AL-SEDAIRY, S. (1994). Effects of cytokine mediated modulation of nm23 expression on the invasion and metastatic behavior of adriamycin-resistant melanoma cells. Int J. Cancer (in press).

POSTEL, E.H., BERBERICH, S.J., FLINT, S.J. & FERRONE, C.A. (1993). Human c-myc transcription factor PuF identified as nm23-H2 nucleoside diphosphate kinase, a candidate suppressor of tumor metastasis. Science, 261, 478-480.

ROSEGAND, A.M., KRUTSCHE, H.C., SHEARM, A., BIGGS, J.R., BARKER, E., MARGULES, I.M.K., KING, C.R., LIOTTA, L.A. & STEEG, P.S. (1989). Reduced Nm23 Awd protein in tumor metastasis and aberrant Drosophila development. Nature, 342, 177-180.

SHI, Y., ZOU, M., SCHMIDT, H., JUHASZ, F., STENSKY, V., ROBB, D. & FARID, N.R. (1991). High rates of ras codon 61 mutation in thyroid tumors in an iodide-deficient area. Cancer Res., 51, 2690-2693.

STAHL, J.A., LEONE, A., ROSEGAND, A.M., PORTER, L., KING, C.R. & STEEG, P.S. (1991). Identification of a second human nm23 gene, nm23-H3. Cancer Res., 51, 445-449.

STEEG, P.S., BELICACQUA, G., KOPPER, L., THORGEIRSSON, U., TALMDGE, J.E., LIOTTA, L.A. & SOBEL, M.E. (1988a). Evidence for a novel gene associated with low tumor metastatic potential. J. Natl Cancer Inst., 80, 200-204.

STEEG, P.S., BELICACQUA, G., POZZATI, R., LIOTTA, L.A. & SOBEL, M.E. (1988b). Altered expression of nm23, a gene associated with low tumor metastatic potential, during adenovirus 2 E1a inhibition of experimental metastasis. Cancer Res., 48, 6550-6554.

STEEG, P.S., BELICACQUA, G., ROSEGAND, A.M., SOBEL, M.E., CIOCE, V. & LIOTTA, L. (1989). Altered gene expression in tumor metastasis: the nm23 gene. In Cancer Metastasis. Molecular and Cellular Biology.Host, Immune Responses and Perspectives for Treatment, Schirmacher, V. & Schwartz-Albiez, R. (eds), pp. 48-52. Springer: Berlin.

WALLET, V., MUTZEL, R., TROLL, H., BARU, O., WURSTER, B., VERON, M. & LACOMBE, M.-L. (1990). Dicystostelium nucleotide diphosphate kinase highly homologous to nm23 and Awd proteins involved in mammalian tumor metastasis and Drosophila development. J. Natl Cancer Inst., 82, 1199-1202.

WALTON, G. & GILL, G. (1975). Nucleotide regulation of a coryn-otic protein synthesis initiation complex. Biochim. Biophys. Acta, 390, 231-245.

YAGEL, S., PARHAR, R.S., JEFFREY, J.J. & LALA, P.K. (1988). Normal nonmetastatic human trophoblast cell lines in vitro invasive properties of malignant cells. J. Cell Physiol., 136, 455-462.

YOUNG, M.R., OKADA, F., TADA, M., HOSOKAWA, M. & KOBAYASHI, H. (1991). Association of increased tumor cell responsiveness to prostat glandulin E2 with more aggressive tumor behaviour: Invasion Metastasis, 11, 48-57.

ZOU, M., SHI, Y., AL-SEDAIRY, S. & FARID, N.R. (1993). High levels of nm23 gene expression in advanced stage of thyroid carcinomas. Br. J. Cancer, 68, 385-388.