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MINIREVIEW

Commentary: *nm23*, a metastasis suppressor gene with a tumor suppressor gene aptitude?

Daniela Lombardi

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nm23 debut

Seven murine K-1735 melanoma derived cell lines, each endowed with different metastatic potential, were compared by differential colony hybridization leading to the identification of the first *nm23* gene (Steeg et al., 1988). The isolated *nm23* cDNA clone was poorly expressed in highly metastatic cell lines as compared to low metastatic counterparts, suggesting that the *nm23* gene was modulated in the invasive cells. Under a more intriguing point of view, the finding provided the first evidence that a specific gene might negatively regulate the invasive phenotype thus envisaging the existence of metastasis suppressor genes.

Since that contribution, many other genes known to participate in several different cell processes have been also proposed as metastasis suppressors (Steeg, 2004). In general, the mechanisms by which they control metastasis formation are still under investigation.

Common features of metastasis suppressor genes

A widely accepted definition states that a metastasis suppressor gene inhibits the spread of cancer cells to secondary sites without affecting tumorigenicity. Reduction or loss of expression correlates with tumor progression and metastases. Hence, the metastatic potential of a malignant tumor depends on metastasis suppressors expression and function. Typically, significant reduction of invasive capabilities *in vitro* and metastatic potential *in vivo* of tumor cell lines follows re-expression of *bona fide* metastasis genes whereas, cell proliferation *in vitro* and tumor size *in vivo* are not affected.

Features of *nm23*

Despite extensive studies, *nm23* functions are far from being completely elucidated. Nevertheless, it appears evident that the *nm23* gene exerts a multifunctional role in a variety of biological processes, suggesting that it might be considered more than a mere metastasis suppressor.

Metastases

It has been reported an inverse correlation between *nm23* expression and the metastatic potential of several human solid tumors (Hartsough and Steeg, 2000; Ouatas et al., 2003). The metastasis suppressive activity of murine *nm23*-M1 and human *nm*23-H1 homologue genes has been extensively documented by experimental data. Of note, when over-expressed, they significantly decreased *in vitro* cell motility, an essential event in metastasis spreading. Moreover, re-expression of the *nm23* genes in different invasive tumor cell lines resulted into a reduction of metastases formation *in vivo* (Ouatas et al., 2003; Steeg, 2004).

Differentiation

Many studies have demonstrated the involvement of the *nm23* genes in cell differentiation. Transduction of

D. Lombardi (✉)
Department of Experimental Medicine, University of L'Aquila, Coppito 2, 67100 L'Aquila, Italy
e-mail: dlombardi@cc.univaq.it

nm23-H1 cDNA in MDA-MB-435 mammary carcinoma cells induced the formation of organized acinus-like structures and the synthesis and secretion of basement membrane components in a three-dimensional matrix (Howlett et al., 1994). The link with mammary differentiation has also been supported by studies performed in *nm23*-M1 knockout mice in which mammary gland development was largely impaired (Arnaud-Dabernat et al., 2003). Moreover, at the onset of mouse organogenesis an increased expression of *nm23*-M1 was generally displayed by organs of epithelial origin and was particularly remarkable in the neural system (Lakso et al., 1992; Gervasi et al., 1998). Up-regulation of *nm23*-R1 expression was observed upon induction of differentiation in rat C6 glioma cells (Roymans et al., 2000). Further, rat PC12 pheochromocytoma cells have been largely used as an *in vitro* model system to evaluate *nm23* involvement in neural differentiation (Gervasi et al., 1996; Ishijima et al., 1999; Lombardi et al., 2001). Over-expression of *nm23*-M1 or *nm23*-H1 in PC12 greatly enhanced differentiation in response to NGF *via* early induction of cytoskeleton proteins specific to neuritegenesis and consequent rapid neurite elongation (Gervasi et al., 1996; Lombardi et al., 2001).

Cell cycle

Cell cycle withdrawal is a prerequisite for terminal cell differentiation. MDA-MB-435 mammary carcinoma cells transduced with *nm23*-H1 showed a reduced growth rate (Howlett et al., 1994). *nm23*-M1 over-expressing PC12 cells exposed to NGF rapidly underwent growth arrest accumulating in the G0/G1 phase of cell cycle in response to NGF treatment. On the contrary, PC12 cells in which *nm23*-M1 expression was inhibited by anti-sense cDNA transduction displayed a remarkably higher proliferation rate and did not differentiate despite NGF treatment (Gervasi et al., 1996). Moreover, besides the above-mentioned suppressive effects on proliferation by ectopic expression of *nm*23, a remarkably increased expression of the endogenous *nm23* gene product has been reported in terminally differentiated PC12 cells and rat C2C12 muscle cells (Gervasi et al., 1996; Lombardi et al., 1995). These findings prompt the consideration that *nm23* promotes differentiation by interfering with cell cycle progression. It is well known that normal cell cycle regulation is driven by interplay of proteins that signal cell to divide and proteins that prevent cell division. The latter restraining proteins perform cell cycle quality control at defined checkpoints. Their function depends on enhanced protein stability or up-regulation whereas is impaired by posttranslational modifications, such as phosphorylation and/or ubiquitination and by mutation dependent loss of function. The so-called tumor suppressor genes encode such cell cycle negative regulators and their deletion or inactivating mutations contribute to cancer development. Noteworthy, expression of *nm23*-M1 or *nm23*-H1 induced *Rb2*/*p130* promoter activity and accumulation of the active hypophosphorylated pRb2/p130 protein in PC12 cells (Lombardi et al., 2001). Of note, *Rb2*/*p130* belongs to the family of the retinoblastoma (*RB*) gene, the prototype of the tumor suppressor genes that negatively control the G1/S phase transition, and are also crucial effectors in cell differentiation (Lipinski and Jacks, 1999; Nguyen and McCance, 2005). In this context, it may be conceivably proposed that *nm23* plays tumor suppressor-like functions in inhibiting cell cycle progression and therefore inducing differentiation.

Apoptosis

Noteworthy, the Nm23-H1 protein has been identified as the Granzyme A (GzmA)-activated DNase responsible for a caspase-independent apoptosis pathway elicited by cytotoxic T lymphocytes (CTL) in virus-infected or tumor cells (Fan et al., 2003). The finding appears very intriguing since suggests that Nm23-H1 expression levels in caspase-resistant transformed cells can be relevant to successful immune surveillance, a basilar step in the prevention of primary tumors development. Therefore, the participation of Nm23-H1 in the apoptotic process outlines a role for this protein also in the early phases of tumorigenesis.

DNA repair

It has been reported that purified recombinant Nm23-H1 protein exhibits nuclease activity *in vitro* with characteristics of 3′-5′ exonuclease (Ma et al., 2004). The 3′-5′ exonuclease activity is strictly linked to DNA repair, a cellular function that prevents genomic instability and, therefore obstacles malignant transformation and progression. An actual role of Nm23-H1 in DNA repair *in vivo* is still under investigation. Anyway, promising preliminary results obtained using yeast and mammalian cell lines corroborate the hypothesis (Kaetzel et al., this issue).

Interactions with virus oncoproteins

It has been shown that Epstein-Barr virus (EBV) EBNA-3C and EBNA-1 latent nuclear antigens, involved in cell immortalization and initiation of transformation, interact with the Nm23-H1 protein. (Subramanian et al., 2001; Murakami et al., 2005). Moreover, an interaction between the Human Papillomavirus-16 (HPV-16) E7 oncoprotein and Nm23-H1 has been recently identified. In a keratinocyte cell line expressing HPV-16 E7, such interaction led to down-modulation and inactivation of the endogenous Nm23-H1 protein and, as a consequence, to increased proliferation rate, impaired differentiation, acquirement of resistance to GzmaA-induced apoptosis and extracellular matrix invasion

properties (Mileo et al., this issue). HPV-16 is linked to more than 90% of cervical cancers and 50% of other ano-genital cancers in humans (zur Hausen, 2002; Munoz et al., 2003). HPV-16 primary transforming mechanisms has been so far identified in the viral E7 oncoprotein binding to and destabilization of the tumor suppressor pRb protein (Munger et al., 2001). Effects of the interactions between HPV-16 E7 and Nm23-H1 besides suggesting a novel transforming strategy of the viral oncoprotein, remarkably, also delineates a wider scenario as far Nm23 function in tumor onset and progression. Actually, targeting of Nm23-H1 by HPV-16 E7 not only affects cell invasiveness but also cell cycle, differentiation and apoptosis, thus corroborating the hypothesis that Nm23 impairment might be crucial at multiple stages during tumor development.

Concluding remarks

It is emerging that *nm23* biological role goes far beyond the borders that outline the range of action of a metastasis suppressor gene. Whereas metastasis suppressors do not affect cell growth, *nm23* expression inhibits cell cycle progression and, as a consequence, drives cell differentiation thus functionally behaving as tumor suppressor genes. Metastasis spreading is a late event in tumor progression; nevertheless the Nm23-H1 protein is recognized as the DNase responsible for an apoptosis pathway crucial for immune prevention of primary tumors growth. Increased overall mutation rates characterize early steps in tumor progression (Jackson and Loeb, 1998). Nm23-H1 3′-5′ exonuclease activity envisages a hypothetical role in maintaining genomic stability. The interference of the HPV-16 E7 oncoprotein with the *nm23*-H1 gene product strongly supports the hypothesis that Nm23-H1 impairment is functional to both malignant transformation and progression.

Differently from tumor suppressors that undergo loss of function by inactivating mutations and more frequently by deletions in cancer cells, *nm23* is inactivated by down modulation except for aggressive neuroblastoma, where *nm23*-H1 is amplified (Leone et al., 1993) downstream the amplification of the N-*myc* oncogene (Godfried et al., 2002; Valentijn et al., 2005). In fact, *nm23*-H1 over-expression depends on its banding to 17q21 in the chromosome 17q region that is over-represented in N-*myc* amplified neuroblastomas (Bown, 2001). Anyway, amplified *nm23*-H1 is affected by the S120G mutation (Leone et al., 1993; Chang et al., 1994) that is known to impair the folding of the gene product (Lascu et al., 1997) and the formation of protein examers strictly required for proper function and interaction with other factors (Chang et al., 1996). Noteworthy, S120G mutated *nm23*-H1 is impaired in inhibiting cell invasiveness (MacDonald et al., 1996), in promoting cell differentiation and inducing the expression of the tumor suppressor *Rb2*/*p130* gene (Lombardi et al., 2001). This scenario suggests nm23-H1 loss of function in aggressive neuroblastoma.

In view of the above, the *nm23* suppressive function is clearly occurring at multiple distinct stages of tumor development and progression. The *nm23* gene product has been found to be involved in several protein-protein interactions (Lombardi and Mileo, 2003). Further studies in this direction are likely to provide evidence of novel partners and signaling pathways, thus allowing a better understanding of *nm23* multifaceted role in controlling cell transformation.

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