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nm23 is expressed in reactive mesothelium and is not useful for detection of malignant cells in serous effusions

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Abstract. The cytological dilemma of distinguishing malignant cells from proliferating mesothelium has generated volumes of research but no clear consensus regarding an optimal staining panel. nm23 has been frequently described in malignant cells as a metastasis suppressor, but its mechanism of action and the relationship between nm23 expression, metastases, and prognosis is controversial. This is the first study to apply nm23 immunostaining in the setting of effusion cytology. One hundred samples of effusions (56 malignant and 44 benign) were immunostained with nm23 using the biotin-avidin technique and diaminobenzidine as a chromogen. Additionally, a mucicarmine stain was performed on most samples, all of which were evaluated for nm23 expression and mucin in a blinded fashion. After all the samples were reviewed, the diagnoses were disclosed and staining patterns evaluated. From the malignant cases, 51 of 56 cases were positive for nm23 in at least 5% of malignant cells, while 27 of 44 reactive mesothelium cases were also positive in at least 5% of cells. Of the malignant cases, all non-adeno-carcinomas expressed nm23. We conclude that nm23 is a highly sensitive marker for detecting malignant cells. Its relatively high rate of expression in reactive mesothelium supports previous studies that suggest nm23 is expressed in proliferating cells.

Introduction

The cytologic detection of the carcinoma in serous effusions is typically a straightforward diagnosis, but difficulties can arise in the setting of reactive or inflamed mesothelium. Numerous studies have been published in an attempt to define an optimal immunostain or immunostain panel to help resolve difficult cases. To date, no consensus has been reached in this regard (1-4). Other diagnostic adjuncts such as *in situ* hybridization and electron microscopy have also been studied with no widespread acceptance (5-7).

Nucleoside diphosphate kinases are a family of enzymes engaged in the production of nucleoside triphosphates other than ATP. One such enzyme is nucleoside diphosphotase A or nm23. nm23, particularly the H1 isoform, is also considered both as a metastasis suppressor gene and as a cell growth regulatory gene. The enzyme also performs several other roles in the cell function but not all of its substrates have been identified. It is unclear exactly how metastases are inhibited by nm23, but suppression may be linked to the down regulation of cell growth of metastatic deposits (8). The expression of this enzyme has been inconsistently associated with survival, incidence of lymph node metastasis or cell proliferation in breast and head and neck carcinomas (8-11). There are also conflicting reports regarding metastasis suppression and nm23 expression in other common carcinomas including those of the lung and colon (12,13). Another item of interest is the differential expression of nm23 between benign and malignant tissue and its role in cell proliferation. For example, as detected by immunocytochemistry, ovarian malignancies express nm23 more often than their benign counterparts. However, both tumor types will express nm23 and this antigen is not a means of discriminating benign from malignant ovarian tumors (14,15).

There is one study which has examined the expression of nm23 in ascites fluid from patients with ovarian carcinoma and other ovarian neoplasms (14). However, no study has specifically addressed the potential of the nm23 immunostain for detecting malignant cells in serous effusions. We examined nm23 as a diagnostic adjunct in this cytologic setting.

Materials and methods

One hundred cases were selected from the archives of the Presbyterian Medical Center and the Hospital of the University of Pennsylvania from 1998 to 2003. Many of these cases have been reported previously (16-19). Forty-four cases were diagnosed as benign reactive mesothelium; the remaining 56 cases were malignant. The malignant cases were primarily adenocarcinomas with a small number of other carcinomas and mesotheliomas. The case distribution is outlined in Table I.

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Table I. Distribution of cases studied with the nm23 immunostain by diagnosis and source.

Diagnosis (number)	Anatomic source of specimen		
	Pleural	Peritoneal	Pericardial
Benign conditions			
Heart (8)	5	1	2
Lung (3)	3	0	0
Gastrointestinal (3)	3	0	0
Other (6)	4	0	2
None/unknown (24)	16	8	0
Total benign (44)	31	9	4
Adenocarcinoma			
Breast (5)	4	1	0
Lung (11)	8	1	2
Renal cell (4)	3	1	0
Other (12)	7	5	0
Unknown (14)	9	3	2
Total (46)	31	11	4
Mesothelioma (6)	4	2	0
Neuroendocrine (2)	2	0	0
Squamous cell (2)	2	0	0
Total malignant (56)	39	13	4

Table II. Staining characteristics of serous effusions immunostained with nm23.

Diagnosis (number)	nm23 positive (%)	Mucin positive (%)
Benign (44)	27 (61)	0
Adenocarcinoma (46)	41 (89)	18 (39)
Breast (5)	3 (60)	2 (40)
Lung (11)	10 (91)	4 (36)
Renal cell (4)	4 (100)	0
Other (12)	10 (83)	2 (29)
Unknown (13)	11 (85)	9 (69)
Mesothelioma (6)	6 (100)	0
Neuroendocrine (2)	2 (100)	0
Squamous cell (2)	2 (100)	0

All cases were selected using the following criteria: an unequivocal diagnosis of malignancy or benignity, patients with a benign diagnosis had no history, clinical or radiological suspicion of malignancy (diagnoses were rendered based on cellular morphology correlated, when possible, with previous surgical pathology material), and that a cellular cell block containing diagnostic cells on the last H&E slide cut from the block with adequate material remaining in the block was available. All specimens were fixed in formaldehyde prior to embedding in paraffin.

Immunohistochemistry was performed using the avidin-biotin complex method on an Optimax Plus automated immunostainer (BioGenex, San Ramon, CA). Briefly, four micron sections on coated slides were deparaffinized in xylene for 10 min, and rehydrated in 100% and 95% alcohol. Endogenous peroxidase activity was blocked using a hydrogen peroxide block (Dako, Carpenteria, CA) for 10 min. The nm23 antibody (pre-dilute, BioGenex) was incubated at room temperature for 60 min in a humidified environment. A polymer labeled secondary antibody (Dako) was applied for 30 min followed by incubation with diaminobenzidine (Dako) as a chromogen and then counterstained with hematoxylin. Breast carcinoma samples provided by the Co-operative Human Tissue Network (a division of the National Cancer Institute) served as a positive control. Mucin staining was also performed in 36 malignant cases and 28 benign cases using the Mayer's mucicarmine staining technique.

Slides were graded in a blinded fashion for diffuse cytoplasmic staining that was distinct and clearly stronger than background staining. Identifying such staining in at least 5% of tumor cells rendered the case positive. Staining that was not unequivocally distinct from background staining was considered negative, as this was likely to be residual endogenous peroxidase activity.

Results

All cases were successfully stained for nm23. Of the adenocarcinomas, including renal cell carcinomas, 41 of 46 (89%) exhibited immunoreactivity for nm23 in at least 5% of identified cells. Also, 18 of these same 46 (39%) adenocarcinomas were positive for mucin. Together, the combination of nm23 and mucin identified 45 of the 46 (98%) adenocarcinomas studied. Also, all six mesotheliomas, both neuroendocrine carcinomas and both squamous cell carcinomas were positive for nm23 (Fig. 1A). Overall, nm23 was expressed in 51 of 56 (91%) malignant effusions. In contrast, 27 of the 44 (61%) cases of benign reactive mesothelium also exhibited immunoreactivity for nm23 with a range of five to 90% of mesothelial cells staining (Fig. 1B). None of the benign cases exhibited intracellular mucin. These results are summarized in Table II.

Of the adenocarcinomas, 10 of 11 (91%) lung samples were positive for nm23 with a range of 40-90% of tumor cells staining. Breast carcinoma samples exhibited the fewest number of positive cases with three of five (60%) samples being positive with a range of 60-90% of the cells staining. The observation that all malignancies that were not adenocarcinomas exhibited positive staining for nm23 was of interest.

Discussion

Typically, the cytomorphology of mesothelial and carcinomatous cells is sufficiently different from each other to permit an accurate distinction between the two. In the clinical setting of reactive or inflamed mesothelial cells, this distinction can be lost. Reactive mesothelial cells can acquire

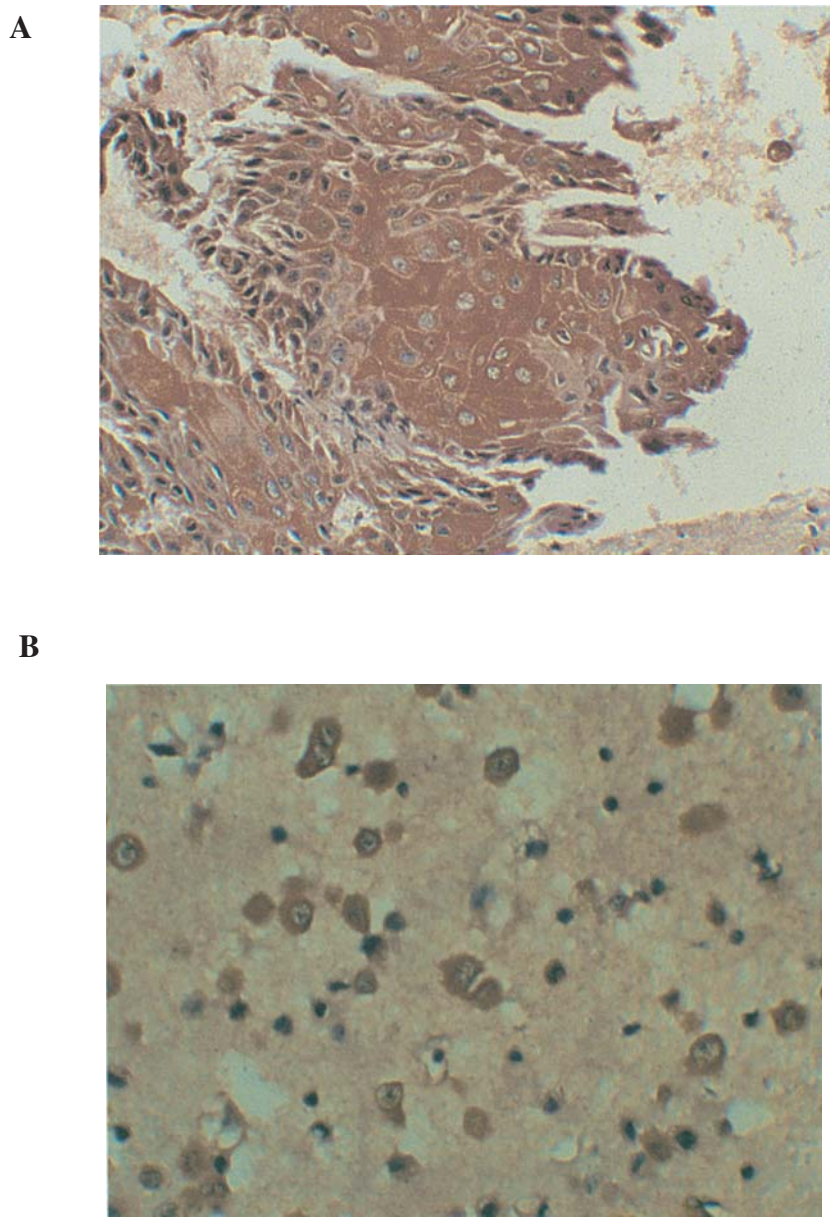

Figure 1. (A) Squamous carcinoma exhibiting immunoreactivity to nm23. Original magnification x200. (B) Reactive mesothelium from a patient with cardiac disease also immunoreactive to nm23. Original magnification x200.

nuclear morphologic features difficult to reliably discriminate from malignant cells. To overcome this diagnostic dilemma, multiple immunostains and other diagnostic adjuncts have been investigated. To date, no firm consensus has been reached regarding an optimal technique or panel.

nm23 is one of several different metastasis suppressor genes; its mechanism of action is not fully understood but nm23 may down regulate the cell growth of metastases. The expression of nm23 in primary tumors has been associated with improved survival in carcinomas of the ovary and head and neck (9). However, there is conflicting data regarding the expression of nm23 in breast carcinoma and its influence on tumor growth (10,11). The expression of nm23 in other carcinomas also shows no consistency between expression and prognosis. Simone *et al* and Harlozinska *et al* have examined nm23 expression in the ascitic fluid of patients with ovarian neoplasms (14,15). Data from these studies suggest that nm23 is detected in benign ascitic fluid as well as that containing ovarian carcinoma. However, these data are confounded by the presence of benign ovarian neoplasms in cases with benign ascites; benign ovarian neoplasms can express nm23 and may have exfoliated such cells into the ascitic fluid (14,15). No other studies have been identified that address the expression of nm23 in serous fluids or its clinical utility in this cytologic setting.

Data presented here suggest that nm23 is usually expressed in a variety of malignancies, and frequently expressed in reactive mesothelium. When combined with mucin staining, nm23 proved to be a highly sensitive immunostain for the detection of adenocarcinomas. As a single stain, nm23 was expressed in all cases of mesotheliomas, renal cell carcinomas, squamous cell carcinomas, and neuroendocrine

carcinomas. However, the number of tumors such as mesotheliomas or squamous cell carcinomas studied here was small and an additional study is needed to properly document nm23 expression. Additionally, nm23 was detected in 61% of benign reactive effusions. Data presented here are consistent with the study conducted by Cipollini and others who reported that nm23 expression correlates with cell proliferation (11,14,15). These data also suggest that nm23 plays a role in mesothelial proliferation as well as in epithelial proliferation.

The rate of nm23 expression is higher than the rates reported in primary or metastatic tumors and in reactive mesothelium. One reason is that the study by Simone *et al* used a cut-off point of 15% of cells staining before considering a case positive, as opposed to 5% for the data presented here. For clinical use, nm23 is not specific enough to discriminate benign from malignant cells. However, these data suggest that nm23 may play a role in cell proliferation in both benign and malignant tissue.

In conclusion, nm23 is frequently expressed in reactive mesothelium and a variety of malignant cells in serous effusions; thus, nm23 is not useful for detecting malignant cells in such specimens. nm23 may play a role in the proliferation of benign and malignant cells.

References

1. Queiroz C, Barral-Netto M and Bacchi CE: Characterizing subpopulations of neoplastic cells in serous effusions: The role of immunocytochemistry. Acta Cytol 45: 18-22, 2001.
2. Osborn M and Wenancjusz D: Special techniques in cytopathology. In: Comprehensive Cytopathology, Marluce Bibbo, 2nd ed. WB Saunders, Philadelphia, pp1058-1060, 1996.
3. Fetch PA and Abati A: Immunocytochemistry in effusion cytology: a contemporary review. Cancer Cytopathol 93: 293-308, 2001.
4. Ferrandez-Izquierdo A, Navarro-Fos S, Gonzalez-Devesa M, Gil-Benso R and Llombar-Bosch A: Immunocytochemical typification of mesothelial cells in effusions: *in vivo* and *in vitro* models. Diagn Cytopathol 10: 256-262, 1994.
5. Saha I, Dey P, Vhora H and Nijhawan R: Role of DNA flow cytometry and image cytometry on effusion fluid. Diagn Cytopathol 22: 81-85, 2000.
6. Dejmek A, Yahata N, Ohyashiki K, Ebihara Y, Kakihane M, Hirano T, Kawate N and Kato H: *In situ* telomerase activity in pleural effusions: a promising marker for malignancy. Diagn Cytopathol 24: 11-15, 2001.
7. Cappellari A, Furlan C, Cardellini P, Bagarella M and Corradi G: Morphologic analysis of effusion cells by scanning electron microscopy. Tumori 78: 10-12, 1992.
8. Graham AJN, Maxwell P, Mulholland K, Patterson AH, Anderson N, McManus KG, Bharucha H and McGuigan JA: Increased nm23 immunoreactivity is associated with selective inhibition of systemic tumour cell dissemination. J Clin Pathol 55: 184-190, 2002.
9. Takes RP, Baatenburg de Jong RJ, Wijffels K, Schuuring E, Litinov SV, Hemans J and van Krieken JHJM: Expression of genetic markers in lymph node metastases compared with their primary tumours in head and neck cancer. J Pathol 194: 198-302, 2001.
10. Ouatas T, Salerno M, Palmieri D and Steeg PS: Basic and translational advances in cancer metastasis: Nm23. J Bioenerg Biomembr 35: 73-79, 2003.
11. Cipollini G, Beryi A, Fiore L, Rainaldi G, Basolo F, Merlo G, Belivacqua G and Caligo MA: Down-regulation of the nm23.h1 gene inhibits cell proliferation. Int J Cancer 73: 297-302, 1997.
12. Gunther K, Dworak O, Remke S, Pfluger R, Merkel S, Hohenberger W and Termond MA: Prediction of distant metastases after curative surgery for rectal cancer. J Surg Res 103: 68-78, 2002.
13. Gunchert VN, del-Rosario A, Kren L, Anwar S, Sheehan CE, Carlson JA and Ross JS: Co-downregulation of PTEN, KAI-1 and nm23-H1 tumor/metastasis suppressor proteins in non-small cell lung cancer. Ann Diagn Pathol 8: 6-16, 2004.
14. Simone G, Galco G, Caponio MA, Campobasso C, De Frenza M, Petroni S, Wiesel S and Leone A: nm23 expression in malignant ascitic effusions of serous ovarian adenocarcinoma. Int J Oncol 19: 885-990, 2001.
15. Harlozinska A, Bar JK and Gerber J: nm23 expression in tissue sections and tumor effusion cells of ovarian neoplasms. Int J Cancer 69: 415-419, 1996.
16. Zimmerman RL and Fogt F: The ß subunit of human chorionic gonadotropin lacks specificity for malignant cells in serous effusions. Mod Pathol 17: 701-704, 2004.
17. Zimmerman RL and Fogt F: Evaluation of the c-Met immunostain to detect malignant cells in body cavity effusions. Oncol Rep 8: 1347-1350, 2001.
18. Zimmerman RL, Goonewardene S and Fogt F: Glucose transporter Glut-1 is of limited value for detecting breast carcinoma in serous effusions. Mod Pathol 14: 748-751, 2001.
19. Zimmerman RL, Fogt F and Goonewardene S: Diagnostic value of a second generation CA15-3 antibody to detect adenocarcinoma in body cavity effusions. Cancer Cytopathol 90: 230-234, 2000.