Expression of nm23 antimetastatic gene product in parathyroid hyperplasia, adenoma and carcinoma

An immunohistological assessment

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ABSTRACT

Objectives: The nm23 gene was initially cloned as a metastasis suppressor gene, but the clinical relevance of nm23 as a metastasis suppressor or prognostic indicator for human cancers remain controversial. To evaluate the role of nm23 protein as a prognostic factor and its role in parathyroid neoplasia, we studied nm23 protein expression by immunohistochemical staining in parathyroid lesions.

Methods: Immunohistochemistry using the avidin-biotin peroxidase complex technique with a polyclonal antibody against the nm23 protein was applied to formalin-fixed, paraffin-embedded tissue specimens obtained from 48 patients. The specimens were collected from 38 patients at the University Health Network, Toronto, Canada and from 10 Saudi patients at the King Abdul-Aziz University Hospital, Jeddah, Kingdom of Saudi Arabia. They included parathyroid carcinomas (5 cases), adenomas (22 cases), hyperplasia (21 cases), and normal parathyroid tissue (10 cases). The immunohistochemistry was completed in 2003 at King Abdul-Aziz University Hospital, Jeddah, KSA and University Health Network, Toronto, Canada.

Results: Expression of nm23 protein was noted in adenomas and carcinomas as well as in hyperplastic parathyroid glands and there was no significant statistical difference between these groups. Normal parathyroid glands did not show any intense immunoreactivity.

Conclusions: The results suggest that expression of nm23 in parathyroid lesions is correlated with tumor proliferation rather than suppression of invasion and metastasis. While our data suggest that nm23 may help in the distinction of normal from proliferative parathyroids, these results do not point to nm23 as a reliable prognostic marker in parathyroid lesions.

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Cancer development and progression is a complex process involving a series of genetic events that result in an imbalance between genes that suppress proliferation and those that promote cell division, disrupt stromal interactions and allow migration. Parathyroid neoplasms, like many other endocrine tumors, are difficult to classify based on morphology alone, and often the distinction between adenoma and carcinoma is based on the presence of invasive behavior and metastases. The nm23 gene was initially cloned as a metastasis suppressor gene whose expression reduced tumor dissemination.[1-4]

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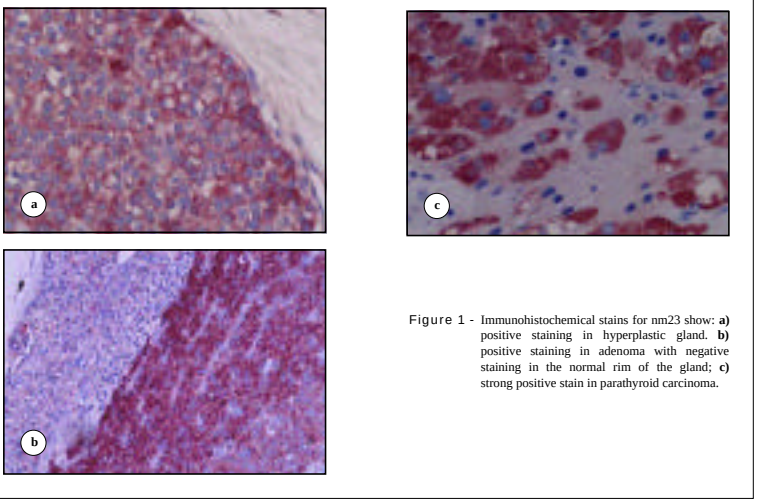

Figure 1 - Immunohistochemical stains for nm23 show: **a)** positive staining in hyperplastic gland. **b)** positive staining in adenoma with negative staining in the normal rim of the gland; **c)** strong positive stain in parathyroid carcinoma.

Quantitative reductions in nm23 messenger ribonucleic acid (mRNA) levels indicating reduced expression of the nm23 gene have been observed in metastatic breast carcinoma,[1,5,6] larynx,[7] lung,[8] salivary glands tumor[9] and ovary. In contrast, nm23 expression does not correlate with metastatic ability in other cancers, including renal,[10,11] thyroid[12] and endocervical carcinoma.[13] The role of nm23 in metastasis of carcinoma of colon is variable.[14-17] A reversed pattern of elevated nm23 mRNA expression has been associated with advanced stage lung and head and neck carcinomas.[18,19] The role of nm23 in the pathogenesis of parathyroid neoplasia and its role in metastasis of those lesions is unknown.

Methods. Specimens obtained retrospectively from 48 patients were as follows: Parathyroids from 38 Canadian patients were collected from the Department of Pathology at the University Health Network, Toronto, Canada. Parathyroid tissue from 10 Saudi patients was collected from the department of Pathology at the King Abdul-Aziz Hospital, Jeddah, Kingdom of Saudi Arabia. They were classified according to accepted criteria[20] as follows: carcinomas (5 cases), adenomas (22 cases), hyperplasia (21 cases), and normal parathyroid tissue (10 cases). Multiple samples were obtained from the same patient in several cases. The nm23 protein was localized on 4 mm sections of formalin-fixed, paraffin-embedded tissue. The avidin-biotin peroxidase complex technique was performed with nm23 polyclonal antibody (DAKO-Denmark) at a dilution of 1:25. Cell proliferation was evaluated using the MIB-1 monoclonal antibody to the Ki-67 antigen (DAKO-Denmark) at a dilution of 1:150. The immunostaining results were assessed by counting the percentage of cells with intense staining. The percentage of nm23 and MIB-1 staining cells was determined in 4 fields at x400 magnification. The Nm23 positivity graded as 0 (0-25% intense staining), +1 (26-50%), +2 (51-75%), and +3 (76-100%). The MIB-1 was scored as positive when more than 1% of the cells were stained.

Result. The age range for the patients was 24-87 years. There were 26 males and 22 females. Normal parathyroid tissues showed lower nm23 expression than neoplastic tissue. None of the normal glands show intense nm23 expression and the rim of normal tissue in adenomatous glands showed no or faint immunoreactivity. Hyperplastic glands showed nm23 intense immunoreactivity in

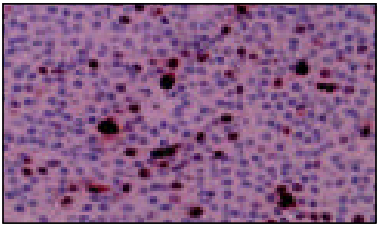

Figure 2 - Immunohistochemical stain for MIB-1 shows a positive nuclear staining in adenoma.

13/21 cases and 14/22 adenomas were positive (**Figure 1**). The nm23 positivity in hyperplastic glands scored as follows; +1 (4 cases), +2 (7 cases), +3 (2 cases). The nm23 positivity in adenomas was scored as follows: +1 (4 cases), +2 (9 cases) and +3 (1 case). All 5 parathyroid carcinomas revealed intense nm23 immunoreactivity (+2 in 2 cases and +3 in 3 cases). All of the hyperplastic and the adenomatous glands showed some positive cells for MIB-1 (**Figure 2**). However, none of those glands show high MIB-1 index (all <3%). The mean percentage of MIB-1 was 1.9 for hyperplastic and 2.2 for adenomatous glands. All the carcinomas contained MIB-1 immunoreactive cells and the mean percentage was 3.9. Two of the 5 cases reveal >5% MIB-1 positive cells. No MIB-1 immunoreactive cells were found in normal parathyroid glands used as controls in the study and there was significant difference in MIB-1 immunoreactivity between hyperplastic/neoplastic glands and normal tissue. The results show that nm23 expression correlates with MIB-1 indices in parathyroid proliferative lesions. Parathyroid carcinoma expressed the highest level of nm23 and had the highest MIB-1 labeling indices. The MIB-1 results are similar to those reported previously.[21-23]

Discussion. Several genes are thought to function as metastasis suppressor genes, including nm23, tissue of metalloproteinase-1 (TIMP) genes, the major histocompatibility complex, and the adenovirus 2 Ela gene product, which has been reported to function as a metastasis suppressor in a rat model of metastasis.[2] In this study, using immunohistochemical localization of nm23, we found no relation between the expression of nm23 and the invasive or metastatic ability of parathyroid neoplasms. The nm23 was expressed in all proliferative lesions of parathyroids, including hyperplastic glands, benign adenomas, and malignant carcinomas. Our results are different from those reported in breast cancer and several other human carcinomas as well as experimental tumors.[1,5-7,14] The nm23 has been found to be associated with cellular proliferation in prostate[24] and thyroid neoplasms.[25] The nm23 gene family is implicated in differentiation and cancer, but the mechanism of this action is unknown. Most nm23 proteins have phosphotransferase [nucleoside diphosphate kinase (NDP kinase)] activity.[26] Nm23 has sequence homology with NDP kinases and has itself been shown to have NDP kinase activity.[27] NDP kinase supply all nucleoside triphosphates except adenosine triphosphate (ATP) to cells and may also participate in signal transduction by supplying guanosine triphosphate (GTP) to G proteins, although any direct association of nm23 with GTP binding protein is questionable. Postel et al[26] suggested that nm23 is involved in deoxyribonucleic acid (DNA) structural transactions necessary for the activity of the c-MYC promoter.[26] Two human nm23 cDNAs have been identified, nm23-H1 and nm23-H2; both predict 17-kD proteins.[2,4] In breast cancer, levels of nm23-H1 expression are inversely correlated with lymph nodes metastasis, and correlate with disease free survival.[28] Transfection of nm23-H1 into breast carcinoma cells suppresses in vivo metastatic potential. However, nm23 expression does not correlate with NDP kinase expression, therefore this mechanism of action is not validated. Further studies of NDP kinase expression in parathyroid lesions will clarify if this may represent a pathogenetic pathway of nm23 in these disorders. The antibody used in this study reacts with both isoforms, nm23-H1 and nm23-H2. A significant correlation between proliferation and nm23-H1 expression was detected in lung carcinomas.[29] Our data are similar in that nm23 expression in parathyroid proliferative lesions correlates with proliferation as identified by MIB-1 labeling. The significance of this particular finding is at present not clear but the data suggest that nm23 may have different roles in the evolution and metastasis of different tumor types.

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