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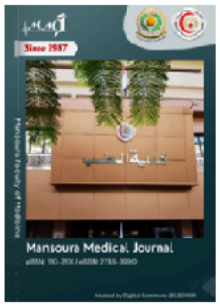

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INVESTIGATION OF THE PROGNOSTIC AND PREDICTIVE VALUE OF NM23 (ANTIMETASTATIC GENE PRODUCT) AND KI-67 IN SAUDI PATIENTS WITH COLORECTAL CARCINOMA: AN IMMUNOHISTOCHEMICAL ANALYSIS

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ABSTRACT

BACKGROUND : Expression of nm23 has been shown to be inversely correlated with the metastatic potential of several human cancers. The objective of this study is to evaluate the value of nm23, and Ki-67 as prognostic markers in patients with colorectal carcinoma

METHODS : We conducted a retrospective analysis on 56 patients with colorectal carcinoma. Immunohistochemistry (IHC) was performed on archival formalin fixed paraffin embedded sections. Antibodies to nm23 and Ki-67 were used. This was correlated with the following clinicopathologic parameters: patient sex, age and survival, stage, and grade of the tumors.

RESULT : Among the 56 cases of colorectal cancer there were 24 female and 32 male. (Male/female=1.3). The mean age was 54.9 years (range 30-80 years). Two of the cases were Dukes' stage A, 28 (stage B), 18 (stage C) and 8 (stage D). Ki-67 was positive in 94%(n=53). The positivity in different stages was as follows (2/2 stage A, 26/28 stage B, 17/18 stage C and 7/8 stage D). Normal mucosa adjacent to cancer showed positive staining for ki-67 in 18/56. nm23 was positive in 84% (n=47) of tumors compared to 45/56 of normal mucosa. The positivity in different stages was

as follows (2/2 stage A, 24/28 stage B, 14/18 stage C and 7/8 stage D).

CONCLUSION : There was no statistical significance difference between normal tissue and tumors regarding nm23 expression, which was not associated with the aforementioned prognostic variables and metastasis in colorectal cancer patients. Although Ki-67 expression expressed strongly in cancer compared to normal tissue ($p<0.05$), there was no relation with survival, grade or Dukes' stage of the tumor.

Key Words : Colorectal carcinoma, nm23, Ki-67, tumor suppressor gene.

INTRODUCTION

nm23 gene was initially cloned as a metastasis suppressor genes for metastasis, whose expression leads to reduction of tumor metastasis [1-4]. There are different results in the literature regarding the significance of nm23 in the metastatic ability of the tumor in different organs. Quantitative reduction in nm23 RNA level and or reduced expression of the nm23 gene have been observed in human metastatic breast carcinoma [1, 5, 6] Hepatocellular carcinoma [7] larynx [8] lung carcinoma[9] and other human cancer. Nm23 was not correlated with metastatic ability in other cancer like renal cell [10,11] thyroid carcinoma[12] endocervical carcinoma[13]. Opposite relationship with high nm23 RNA expression associated with advance stage of lung and head and neck carcinomas[14,15] However the role of nm23 in colorectal neoplasm is controversial and reports of the relationship between the putative metastasis suppressor nm23 and metastasis and/or survival in colorectal cancer patients are conflicting [7,16,17].

MATERIALS AND METHODS

We conducted a retrospective analysis on 56 patients with colorectal carcinoma. All the specimens were routinely fixed in 10% neutral buffered formalin and processed for light microscopic examination. Immunohistochemistry (IHC) was performed on archival formalin fixed paraffin embedded sections. Antibodies to nm23 and Ki-67 were used. This was correlated with the following clinicopathologic parameters: patient sex, age and survival; stage, and grade of the tumors.

RESULTS

Among the 56 cases of colorectal cancer there were 24 female and 32

male. (Male/female=1.3). The mean age was 54.9 years (range 30-80 years). Two of the cases were Dukes' stage A, 28 (stage B), 18 (stage C) and 8 (stage D). Sections that contain tumor with adjacent normal mucosa were selected (figure1). The differentiation of the tumors was as follows: well differentiated 6 cases, moderately differentiated 45 cases, and poorly differentiated 5 cases. Three cases were diagnoses as singnet-ring adenocarcinoma, the other were conventional adenocarcinoma. Nm23 was positive in 84% (n=47) of tumors compared to 45/56 of normal mucosa (figure 2). The positivity in different stages was as follows (2/2 stage A, 24/28 stage B, 14/18 stage C and 7/8 stage D). The positivity of nm23 according to tumor differentiation was as follows: (well differentiated 5/6, moderately differentiated 37/45, poorly differentiated 5/5). Ki-67 was positive in 94%(n=53). The positivity in different stages was as follows (2/2 stage A, 26/28 stage B, 17/18 stage C and 7/8 stage D). The positivity of ki-67 according to tumor differentiation was as follows: (well differentiated 6/6, moderately differentiated 43/45, poorly differentiated 4/5). Normal mucosa adjacent to cancer showed positive staining for ki-67 in 18/56 of the compared to 53/56 of cancer. At the time of reporting 12 patients were dead. Nine of those twelve patients show positive staining for nm23.

Figure 1 : Sections from one of the cases of colorectal carcinoma showing a tumor on the left side and non-neoplastic epithelium on the right side. (Haematoxylen and eosin stain, original power x200).

Figure 2 : Immunohistochemistry of nm23 showing positive staining in the neoplastic and weak staining in non-neoplastic epithelium (original magnification x200).

DISCUSSION

Few genes are reported that might function as metastasis suppressor genes, nm23, tissue inhibitor of metalloproteinase-1 (TIMP-1) gene, the major histocompatibility complex (MHC), and the adenovirus 2 Ela gene product, which may function as a metastasis suppressor in certain rat model system of metastasis[2]. In the current study, in which we used an immunohistochemistry technique using nm23 antibody, we demonstrated that there was no relation between the extent of nm23 gene product and the stage or metastatic ability of colorectal carcinoma. Nm23 was expressed in normal and malignant lesions with no statistical significance difference. Our result disagree with those of several previous breast cancer studies as well as studies in other human carcinomas as well as experimental tumors[1,5-8]. Nm23 has been found to be associated with cellular proliferation in many tumors such as prostate[18], thyroid neoplasm[19] and lung carcinoma[20]. The nm23 gene family in humans is implicated in differentiation and cancer progression, but the biochemical mechanisms are unknown. Most nm23 proteins have phosphotransferase (nucleoside diphosphate kinase) activity[21]. The function of nm23 is uncertain. Nm23 has sequence homology with NDP kinase activity and has it self been shown to have NDP kinase activity [22]. NDP kinase supply all nucleoside triphosphates except ATP to cells and may also participate in signal transudation by supplying GTP to G protein, although any direct association of nm23 with GTP binding protein is questionable. Postel et al suggested that nm23 is involved in DNA structural transactions necessary for the activity of the c-MYC promoter[21]. Two human nm23 cDNA have been reported, nm23-H1 and nm23-H2, both predict 17-kD proteins [2,4]. Patients with low nm23-H1 expression in breast cancer had a significantly greater incidence of lymph nodes metastasis, shorter disease free survival and decreased survival compared with patients with relatively higher nm23-HI1 expression[23]. In addition Transfection of nm23-H1 into breast carcinoma cells suppresses in vivo metastatic potential. The discrepancies regarding the significance of the nm23 gene product/NDP kinase expressions in human malignancy is that the significance of NDP kinase expression may be quite different in different tissue. Quantitative reduction in nm23 RNA level and or reduced

expression of the nm23 gene have been observed in different human metastatic neoplasm[1,5-9]. nm23 was not correlated with metastatic ability in other cancer like renal cell [10,11] and thyroid carcinoma [12], endocervical carcinoma 13. Opposite relationship with high nm23 RNA expression associated with advance stage of lung and head and neck carcinomas [14,15]. However the role of nm23 in colorectal neoplasm is controversial and reports of the relationship between the putative metastasis suppressor nM23 and metastasis and/or survival in colorectal cancer patients are conflicting [7,16,17,24-28]. Some authors demonstrated positive relation of nm23 expression with different prognostic parameters and with metastatic ability in CRC [7,17, 24-26,29]. nM23-H2 expression was not related to survival; however, there was a modest survival advantage with low expression of nM23-H1 17. Dusonchet et al demonstrated that in CRC the expression of the protein is not associated with tumor progression and patient prognosis and suggested that nm23-H1 activity is tissue-specific 27. Garinis et al reported that impairment of nm23-H1 expression is an early event into the progression of colorectal metastasis may therefore play an important role in suppressing the early steps of metastasis in sporadic cases of colorectal carcinomas 24. Other authors suggested also some relation between nm23 and clinical or histopathological prognostic parameters 25, 26. In the current study we used polyclonal antibody against nm23 gene product. However, we need further study to determine correlation between transcriptional and transnational levels of the nm23 gene using antiisoform specific antibodies because the antibody used in this study could react with both isoform of nm23-H1 and nm23-H2. Our study showed that there was a highly significant difference in ki-67 immunoreactivity between normal tissue and malignant tissue. In conclusion, this immunohistochemical analysis shows that the nm23 gene product in colorectal neoplasm is expressed independently of clinicopathological parameters. There is no correlation between expression level and malignant transformation. This confirm that nm23 is not always associated with indicators of tumor malignancy such metastatic potential or prognosis. Thus, nm23 gene is unlikely to be useful as a prognostic indicator, in contrast of previous results in breast carcinoma and other human cancer.

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