HMGA1 Protein Overexpression in Human Breast Carcinomas: Correlation with ErbB2 Expression

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ABSTRACT

We measured, by immunohistochemistry, HMGA1 protein expression in 212 breast tissue specimens: 6 normal samples, 28 hyperplastic lesions (13 with cellular atypia), 11 fibroadenomas, 10 in situ ductal carcinomas, 144 ductal carcinomas, and 13 lobular carcinomas. HMGA1 was not expressed in normal breast tissue; HMGA1 staining was intense in 40% of hyperplastic lesions with cellular atypia and in 60% of ductal carcinomas and weak in fibroadenomas and in hyperplastic lesions without cellular atypia. Because HMGA1 expression was similar among ductal breast carcinomas with different histologic grading, we evaluated the association between HMGA1 expression and that of other markers of breast carcinoma invasion (estrogen and progesterone receptors, Ki-67 antigen, and ErbB2) in 21 cases of grade 3 breast ductal carcinomas and 7 cases of breast lobular carcinomas. We found that HMGA1 expression tended to be associated only with c-erbB-2 expression (Spearman rho: 0.36; P = 0.065). Taken together, these results suggest that HMGA1 expression might be a novel indicator for the diagnosis and prognosis of human breast cancer.

INTRODUCTION

The HMGA protein family is constituted by proteins HMGA1a, HMGA1b, and HMGA2. The first two are encoded by HMGA1 through alternative splicing (1, 2), whereas HMGA2 is encoded by HMGA2 (3). By interacting with the transcription machinery, HMGA1 proteins alter the chromatin structure and thereby regulate the transcriptional activity of several genes (4). HMGA proteins seem to play their major physiologic role during embryonic development. In fact, HMGA1 expression is very high during embryogenesis and negligible in normal adult tissues (5, 6). HMGA1 overexpression was first documented in transformed thyroid cells (7, 8), then in experimental and human carcinomas (9), and subsequently in human thyroid (10, 11), colorectal (12–14), pancreatic duct cell (15), uterine (16), and ovarian (17) cancers.

HMGA overexpression is a feature of tumorigenesis and not merely a result of cell transformation. In fact, when rat thyroid cells, whose HMGA gene expression is blocked by an antisense HMGA1 or HMGA2 cDNA construct, are infected by transforming murine retroviruses, they are not able to grow in soft agar or to form tumors when injected into athymic mice (18, 19). In contrast, untransfected rat cells readily form colonies in soft agar after retroviral infection and are highly tumorigenic on transplantation. Moreover, HMGA1 is abundantly expressed in several human carcinoma cell lines, and blockage of its synthesis by infection with an adenovirus carrying HMGA1 antisense sequences suppressed their growth (20).

Breast cancer is the most common cancer and the second leading cause of cancer mortality in women with about one in nine being affected during their life. Neoplastic breast diseases range from benign fibroadenoma to very aggressive undifferentiated carcinoma. Growth factors and their receptors, intracellular molecules, regulators of cell cycling, and proteases have all been shown to be altered in sporadic breast cancer (21) and ErbB2 overexpression, and a loss of estrogen receptors (ERs), correlates with a poor prognosis.

The aim of our study was to investigate whether HMGA1 protein expression might be an indicator for the diagnosis and prognosis of human breast cancer. This possibility was well supported by previous studies showing that high levels of HMGA1 mRNA are directly correlated with metastatic progression among several mouse mammary tumor cell lines (22). Moreover, both in vitro and in vivo HMGA1b protein binds to and inhibits the activity of both human and mouse BRCA1.
promoters and that an inverse correlation between HMGAI and BRCA1 mRNA and protein expression was found in human mammary carcinoma cell lines and tissues (23). Furthermore, it has been demonstrated that human breast epithelial cells harboring tetracycline-regulated HMGAI transgenes acquire the ability to form both primary and metastatic tumors in nude mice only when the transgenes are actively expressed (24). All of these data would suggest an important role of the HMGAI overexpression in the process of breast carcinogenesis.

In the study reported here, HMGAI protein was detected in 60% of ductal carcinomas and in almost all of the lobular carcinomas. In addition, HMGAI expression was similar among ductal breast carcinomas with different histologic grading but tended to be correlated with c-erbB2 amplification.

MATERIALS AND METHODS

Collection of Breast Tissue Samples. A total of 212 breast tissue specimens constituted by 6 normal breast tissues, 28 hyperplastic lesions (13 with atypical cells), 11 fibroadenomas, 10 in situ ductal carcinomas, 144 ductal carcinomas, and 13 lobular carcinomas were collected at the Istituto Nazionale dei Tumori di Napoli (Naples, Italy) from patients undergoing surgery for breast cancer. All tissue samples were fixed immediately after surgical removal in 4% paraformaldehyde in PBS. The criteria for inclusion in the study were that the routinely processed paraffin blocks were suitable for immunohistochemistry and adequate clinical information.

Immunohistochemical Analysis. Paraffin sections (5 to 6 μm) were deparaffinized, placed in a solution of absolute methanol and 0.3% hydrogen peroxide for 30 minutes and then washed in PBS before immunoperoxidase staining. For ER, progesterone receptor (PgR), and Ki-67 staining, slides were incubated overnight at 4°C in a humidified chamber with ER monoclonal antibody (1:50 dilution; Dako A/S, Glostrup, Denmark), PgR monoclonal antibody (1:50 dilution; Dako), or Ki-67 monoclonal antibody (1:20 dilution; Dako). For ErbB2, the slides were stained with the Dako Herceptin Test kit (Dako), which includes a monoclonal mouse antibody against the cytoplasmic domain of the human ErbB2 protein. For HMGAI immunostaining, the antibodies used in this study were raised against the synthetic peptide SSSKQQPLASKQ specific for the HMGAI proteins (10). They were affinity purified against the synthetic peptide. The slides were subsequently incubated with biotinylated goat antirabbit/antimouse IgG for 20 minutes (Dako LSAB2 System) and then with streptavidin horseradish peroxidase for 20 minutes. For immunostaining, the slides were incubated in 3,3′-diaminobenzidine (Dako) solution containing 0.06 mmol/L 3,3′-diaminobenzidine and 2 mmol/L hydrogen peroxide in 0.05% PBS (pH 7.6) for 5 minutes. After chromogen development, the slides were washed, dehydrated with alcohol and xylene, and mounted with coverslips with Permount mounting medium (Proscitech, Kirwan, Australia). Micrographs were taken on Kodak Ektachrome film with a photo Zeiss system. For the clinicopathological study of ER, PgR, Ki-67, and HMGAI expression, slides were assigned one of four staining scores: 0, no stained cells; 1+, <10% of stained cells; 2+, 11 to 50% of stained positive cells; 3+, 51 to 75% of stained cells; and 4+, 76 to 100% of stained cells. For the ErbB 2 expression study, the proportion of malignant cells stained was scored from 0 to 3: 0, no stained cells; 1+, <10% of weak, focal membrane staining of cancer cells; 2+, moderate, completely staining of membrane of cancer cells; and 3+, strong completely staining of membrane in >10% of cancer cells (Food and Drug Administration approved).

Statistical Analysis. The Fisher’s exact test was used to analyze intrascore differences of HMGAI staining among histologic types of breast tissue and interhistologic grading differences of HMGAI-positive cases among ductal breast carcinomas. One-way ANOVA was used to analyze interhistologic grading differences of percent HMGAI-positive cells among ductal breast carcinomas. The nonparametric Spearman correlation was used to analyze the association between HMGAI
staining score and that of ER, ErbB2, Ki-67, and PgR in breast ductal and lobular carcinomas. Data were analyzed using standard statistical software (SPSS version 9, Chicago, Illinois). 

RESULTS

We measured the expression of the HMGA1 proteins in 212 breast tissues (6 normal tissues, 13 hyperplasias with cellular atypia, 15 hyperplasias without cellular atypia, 11 fibroadenomas, 10 in situ ductal carcinomas, 144 ductal carcinomas, 13 lobular carcinomas, 157 ductal lobular carcinomas). ER, ErbB2, Ki-67, and PgR were detected using immunohistochemistry.

Table 1. HMGA1 protein expression in normal and neoplastic breast tissues by immunohistochemistry.

<table>
<thead>
<tr>
<th>Histological type</th>
<th>HMGA1 staining score * n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Normal breast tissue (n = 6)</td>
<td>6</td>
</tr>
<tr>
<td>Hyperplasias of the ductal epithelium without cellular atypia (n = 13)</td>
<td>9</td>
</tr>
<tr>
<td>Hyperplasias of ductal epithelium with cellular atypia (n = 15)</td>
<td>9</td>
</tr>
<tr>
<td>Fibroadenomas (n = 11)</td>
<td>6</td>
</tr>
<tr>
<td>In situ ductal carcinomas (n = 10)</td>
<td>5</td>
</tr>
<tr>
<td>Ductal carcinomas (n = 144)</td>
<td>58</td>
</tr>
<tr>
<td>Lobular carcinomas (n = 13)</td>
<td>1</td>
</tr>
<tr>
<td>Ductal + lobular carcinomas (n = 157)</td>
<td>59</td>
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</tbody>
</table>

* HMGA1 staining score was as follows: 0, no positive cells; +, positive cells; 1+, <10% of positive cells; 2+, 11–50% of positive cells; 3+, 51–75% of positive cells; 4+, 76–100% positive cells. N, number of cases.

The Fisher’s exact test was used to analyze intracore differences of HMGA1 staining among breast tissues histological types: †P < 0.05 and ‡P < 0.01 versus normal breast tissue; §P < 0.01 and ¶P < 0.05 versus hyperplasias of the ductal epithelium without cellular atypia.

Fig. 2 Immunohistochemical analysis of HMGA1 expression in dysplastic and neoplastic breast tissues. Paraffin sections from dysplastic and neoplastic breast tissues were analyzed by immunohistochemistry with antibodies raised against a specific HMGA1 peptide. A, No immunoreactivity appeared in normal tissue (original magnification, ×100) or (B) in epithelial hyperplasia without cellular atypia (original magnification, ×200). C, nuclear staining in epithelial hyperplasia without cellular atypia (original magnification, ×100). D, intense nuclear and cytoplasmic staining in epithelial hyperplasia with cellular atypia (original magnification, ×200). E, weak staining in a fibroadenoma (original magnification, ×200). F, no immunoreactivity in a fibroadenoma (original magnification, ×100).
adenomas, 10 in situ ductal carcinomas, 144 ductal carcinomas, and 13 lobular carcinomas) with immunohistochemistry with antibodies raised versus the NH2-terminal region of the HMGA1 proteins (10). These antibodies are not able to detect the product of the other member of the HMGA family that is the HMGA2 gene.

The specificity of the antibody is shown in Fig. 1: a positive nuclear staining was present in a breast carcinoma sample immunostained with the HMGA1 antibody (Fig. 1A); conversely, no staining was observed when the same carcinoma sample was stained with antibodies preincubated with the peptide against which antibodies were raised (Fig. 1B). Equally, there was no staining in the absence of the primary antibodies (Fig. 1C).

The data of this analysis are summarized in Table 1. No HMGA1 staining was observed in the normal breast tissues (Fig. 2A). Conversely, HMGA1 expression was variably detected in neoplastic breast tissues (in 30% of hyperplastic lesions without cellular atypia, 40% of hyperplastic lesions with cellular atypia, 45% of fibroadenomas, 50% of in situ ductal carcinomas, 60% of ductal carcinomas, 92% of lobular carcinomas, and 62% of ductal + lobular carcinomas). In particular, HMGA1 expression was significantly more frequent in ductal and lobular carcinomas compared with normal breast tissue, either alone ($P < 0.05$ and $P < 0.01$, respectively) or in combination ($P < 0.01$), and in lobular carcinomas compared with hyperplastic lesions without cellular atypia, either alone or in combination with ductal carcinomas ($P < 0.01$ and $P < 0.05$, respectively). In addition, HMGA1 staining was low-to-intermediate (1 to 50% of positive cells, scores 1+ and 2+) in 100% of hyperplastic lesions without cellular atypia, 80% of fibroadenomas, 60% of in situ ductal carcinomas, and 51% of ductal carcinomas, whereas it was intermediate-to-high (51 to 100% of positive cells, score 3+ and 4+) in 67% of hyperplastic lesions with cellular atypia, 67% of lobular carcinomas, and 51% of ductal + lobular carcinomas.

Representative results are shown Figs. 2 and 3. Fig. 2, B and C, shows epithelial hyperplastic lesions without cellular atypia, which were negative and positive for HMGA1 expression, respectively. Conversely, Fig. 2D shows a positive epithelial hyperplastic lesion with atypical cells; the staining is intense and is comparable with that observed in most of the ductal carcinomas. In Fig. 3, we show two representative carcinomas (one lobular carcinoma and one in situ ductal carcinoma) with H&E (panels A and C) and immunostaining (panels B and D). Nuclear staining was observed only in some of the neoplastic cells of an in situ ductal carcinoma (Fig. 3B), whereas protein HMGA1 was expressed in most of the cells of the lobular breast carcinomas (Fig. 3D). It is noteworthy that in some ductal carcinomas (as also in Fig. 3B) cytoplasmic and nuclear staining was observed. This observation is not new because a similar subcellular location was detected in thyroid and colon neoplasias (11, 14). Expression of very high levels of HMGA1 protein

### Table 2

<table>
<thead>
<tr>
<th>Histological grade</th>
<th>HMGA1-positive cases * n (%)</th>
<th>% HMGA1-positive cells † (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1 (n = 45)</td>
<td>29 (64)</td>
<td>18 ± 15</td>
</tr>
<tr>
<td>Grade 2 (n = 35)</td>
<td>21 (60)</td>
<td>17 ± 6</td>
</tr>
<tr>
<td>Grade 3 (n = 64)</td>
<td>36 (56)</td>
<td>16 ± 3</td>
</tr>
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</table>

* The Fisher’s exact test was used to analyze inter-histological grading differences of HMGA1-positive cases among ductal breast carcinomas, showing no significant difference ($P = $ not significant).
† One-way ANOVA was used to analyze interhistological grading differences of percentage of HMGA1-positive cells among ductal breast carcinomas, showing no significant difference ($P = $ not significant).
An example is shown in Fig. 4, where three G3 ductal carcinomas (panels A, B, and C) showed an increase in HMGA1 expression from the case in panel A to that in panel C. Taken together, these findings indicate a lack of association between HMGA1 expression and morphologic grading in ductal carcinomas.

Therefore, we measured the expression of other indicators of breast carcinoma invasion (ER and PgR receptors, Ki-67, and ErbB2) in 21 ductal and 7 lobular breast carcinomas (Table 3) and analyzed whether their expressions were correlated with that of HMGA1. As shown in Fig. 5, we found that HMGA1 expression tended to be associated only with c-erbB-2 expression (Spearman rho: 0.36; \( P = 0.065 \)). In Fig. 6 we show a representative case of a breast ductal carcinoma (whose H&E staining is shown in panels A and D) that expressed both the HMGA1 and ErbB2 oncogenic proteins (panels B and C, respectively) and a case of a breast ductal carcinoma negative for the two oncogenic proteins (panels E and F).

**DISCUSSION**

The aim of our study was to verify whether HMGA1 protein might be an indicator for the diagnosis and prognosis

![Image](https://example.com/image.png)

**Fig. 4** Immunohistochemical analysis of HMGA1 expression in breast ductal carcinomas (G3). A, immunostaining of a G3 ductal carcinoma with only a few stained cells. B, immunostaining of another G3 ductal carcinoma with more immunostained cells versus the sample shown in A. C, immunostaining of a third G3 ductal carcinoma showing intense immunoreactivity and more immunostained cells versus the samples shown in A and B.

Table 3  HMGA1, ER, PgR, Ki-67, and ErbB2 protein expression in 21 grade 3 breast ductal carcinomas and 7 breast lobular carcinomas

<table>
<thead>
<tr>
<th>Histological type</th>
<th>Histological grading</th>
<th>ER*</th>
<th>PgR*</th>
<th>Ki-67*</th>
<th>c-erbB-2†</th>
<th>HMGA1*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ductal</td>
<td>G3</td>
<td>4+</td>
<td>2+</td>
<td>2+</td>
<td>1+</td>
<td>0</td>
</tr>
<tr>
<td>Ductal</td>
<td>G3</td>
<td>4+</td>
<td>4+</td>
<td>2+</td>
<td>1+</td>
<td>2+</td>
</tr>
<tr>
<td>Ductal</td>
<td>G3</td>
<td>4+</td>
<td>4+</td>
<td>2+</td>
<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td>Ductal</td>
<td>G3</td>
<td>4+</td>
<td>4+</td>
<td>2+</td>
<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td>Ductal</td>
<td>G3</td>
<td>4+</td>
<td>4+</td>
<td>2+</td>
<td>2+</td>
<td>3+</td>
</tr>
<tr>
<td>Ductal</td>
<td>G3</td>
<td>4+</td>
<td>4+</td>
<td>2+</td>
<td>2+</td>
<td>3+</td>
</tr>
<tr>
<td>Ductal</td>
<td>G3</td>
<td>4+</td>
<td>4+</td>
<td>2+</td>
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<tr>
<td>Ductal</td>
<td>G3</td>
<td>4+</td>
<td>4+</td>
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<tr>
<td>Ductal</td>
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<td>4+</td>
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<tr>
<td>Ductal</td>
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</tr>
<tr>
<td>Ductal</td>
<td>G3</td>
<td>4+</td>
<td>4+</td>
<td>2+</td>
<td>2+</td>
<td>3+</td>
</tr>
</tbody>
</table>

* Staining score was, as follows: 0, no positive cells; 1+, <10% of positive cells; 2+, 10–50% of positive cells; 3+, 51–75% of positive cells; 4+, >75% of positive cells.

† Staining score was as follows: (0, no stained cells; 1+, <10% of weak, focal membrane staining of cancer cells; 2+, moderate, completely staining of membrane of cancer cells; 3+, strongly staining of membrane in >10% of cancer cells.

in these samples may account for this result. However, it cannot be excluded at all a biological significance of the HMGA1-cytoplasmic localization.

HMGA1 staining, expressed either as HMGA1-positive cases or as percentage of HMGA1-positive cells, was similar among ductal breast carcinomas with different histologic grading (Table 2). In fact, several cases of ductal carcinomas with the same histologic grading showed different HMGA1 staining.
of human breast cancer. We found that 60% of ductal carcinomas and almost all lobular carcinomas expressed HMGA1 protein versus none of the normal breast tissues. In addition, more than half of these HMGA1-positive cases showed an intermediate-to-high staining score. Conversely, HMGA1 expression occurred in a lower percent of benign lesions and hyperproliferative disorders, showing on average a low-to-intermediate staining score. Noteworthy, although HMGA1 protein was detected only in 40% of hyperplastic lesions with cellular atypia, its staining score was similar to that of carcinomas. Similar results were obtained in atypical colon adenomas (13, 14), suggesting that the induction of the HMGA1 proteins precedes the appearance of the clearly malignant phenotype.

There was no association between HMGA1 expression and histologic grading of ductal carcinomas. In fact, HMGA1 staining differed among malignant neoplastic lesions of the same morphologic grading. This prompted us to investigate whether HMGA1 expression was correlated with other prognostic indicators of breast carcinomas. We found that the staining score for HMGA1 tended to be correlated with that for ErbB2. This is a transmembrane protein with substantial homology to epidermal growth factor receptor. Gene amplification coupled with resultant overexpression of the ErbB2 protein occurs in ~25% of human breast cancers: rapid proliferation and a poor prognosis of breast cancer are associated with c-erbB2 amplification (21). It could be hypothesized that HMGA1 proteins would be able to induce c-erbB2 expression acting on its promoter or, alternatively, that the activation of the ErbB2 transduction pathway may lead to increased HMGA1 protein synthesis. However, the first hypothesis appears unlikely because of the absence of AT-rich regions (where the HMGA1 proteins bind DNA) in the c-erbB2 promoter.

Overexpression of HMGA1 might be critical in the process of breast carcinogenesis. In fact, we previously demonstrated that HMGA1 negatively regulates the expression of the BRCA1 gene, which is mutated in most familial breast carcinomas, and its expression is reduced in the sporadic forms (23). Therefore, the induction of HMGA1 expression in breast carcinoma would down-regulate BRCA1 expression, thereby accounting for the aggressive phenotype. Moreover, HMGA1 expression might up-regulate several genes that are required for the process of invasion and metastasis (24).

It is interesting that a significant number (30%) of ductal breast carcinomas did not express the HMGA1 gene. This result appears to contrast with the finding of HMGA1 expression in all malignant thyroid, colonic, and pancreatic neoplasias studied thus far (11, 14, 15). Two hypotheses might be envisaged to...
account for this discrepancy: (a) different molecular events might be involved in breast carcinomas compared with other neoplasias, and it cannot be excluded that some genetic alterations leading to cancer are not able to induce *HMGA1* gene expression; and (b) other HMGA-related genes may be induced in the process of breast carcinogenesis. Moreover, we cannot exclude a slight increase in HMGA1 expression not detectable by immunohistochemistry. Interestingly, 30% of ovarian carcinomas did not express HMGA1 when evaluated by immunohistochemistry and reverse transcription-PCR (17).

On the basis of our findings, one may envisage therapy of some breast cancers, in particular the most aggressive ones, based on the blockage of HMGA1 functions. This approach would have the advantage of low toxicity and high specificity because HMGA1 expression is low or null in normal adult tissues.

In conclusion, the evaluation of HMGA1 protein levels might be an indicator for the diagnosis and prognosis of human breast cancer.

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