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High mobility group protein HMGA1 expression in breast cancer reveals a positive correlation with tumour grade

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Summary. Members of the HMGA protein (high mobility group protein A) family act as master switches of the chromatin structure by bending DNA and thus modulating the formation of transcription factor complexes of a number of target genes. Accordingly, HMGA proteins have been shown to be associated with the development and/or progression of a variety of benign and malignant tumours. Nevertheless, the HMGA1 expression studies published so far have not included primary breast cancer samples. In this study we have investigated the HMGA1 expression patterns in a series of 170 breast cancer samples by immunohistochemistry. We have found a strong variation in HMGA1 expression between the tumours. Based on an immunoreactive score (IRS) 14.1% of the tumour samples were scored to IRS 8-12 (strong positivity for HMGA1), 24.7% were scored to IRS 4-6 (moderate positivity), 25.3% were scored to IRS 1-3 (weak positivity), and 35.9% showed no positivity at all. Immunoreaction could be detected in all histological types of breast cancers analysed with the exception of invasive papillary and cribriform carcinoma. Statistical analysis revealed a strong correlation between tumour grade and HMGA1 expression ($rs=0.3516$, $p<0.0001$). Thus, the HMGA1 expression level can be considered a potential prognostic marker for breast cancer.

Key words: High mobility group proteins, HMGA1 expression, Breast cancer, Prognostic marker, Immunoreactive score (IRS)

Introduction

High mobility group proteins are small chromatin-associated nonhistone proteins which have been subdivided into three families because of their functional sequence motives: the HMGB, HMGN, and HMGA protein family (Bustin, 2001). Members of the HMGA family act as master switches of the chromatin structure by bending DNA, enabling or disabling the formation of transcription factor complexes of a number of target genes (for review: Liu et al., 2001; Reeves and Beckerbauer, 2001). The HMGA family consists of two genes, i.e. *HMGA1* and *HMGA2*. *HMGA1* has been assigned to the chromosomal band 6p21.3 and encodes for HMGA1a and HMGA1b (formerly known as HMGI and HMGY) by alternative splicing (Friedmann et al., 1993). In adult tissues an *HMGA1* expression is only detectable at very low levels or is even absent, whereas it is abundantly expressed in embryonal cells (Chiappetta et al., 1996). Very often, 6p21 is affected by aberrations leading to an upregulation of *HMGA1* in benign mesenchymal tumours, e.g. lipomas, uterine leiomyomas, pulmonary chondroid hamartomas, and endometrial polyps (Williams et al., 1997; Kazmierczak et al., 1998; Tallini et al., 2000). Transcriptional activation due to a chromosomal alteration of *HMGA1* is probably an early and often even primary event. In contrast, *HMGA1* expression in malignant epithelial tumours seems to be a rather late event associated with an aggressive behavior of the tumours. Thus, an overexpression of *HMGA1* was reported for a number of malignancies including thyroid, prostatic, pancreatic, uterus cervical, and colorectal cancer (Tamimi et al., 1993; Chiappetta et al., 1995, 1998; Fedele et al., 1996; Bandiera et al., 1998; Abe et al., 1999, 2000). The correlation between HMGA1 expression and tumour aggressiveness in some of these malignancies has led to the conclusion that HMGA1 expression may present a powerful prognostic molecular marker. The causal role of HMGA1 expression in the progression of carcinomas has been elucidated by a set of in vitro experiments involving *HMGA1* sense and antisense transfection assays (Wood et al., 2000a,b; Reeves et al., 2001). A proof of concept for a therapy aimed at the downregulation of HMGA1 protein in tumours has been presented by Scala et al. (2000) who were able to show

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that an *HMGA1* antisense strategy using an adenoviral vector treatment of tumours induced in athymic mice caused a drastic reduction in tumour size. However, despite the emerging role of *HMGA1* gene expression in malignancies, there are, to the best of our knowledge, no studies on the HMGA1 expression in primary breast cancer samples. Thus, in the present study we have analysed 170 breast cancer samples by immunohistochemistry to find out whether or not HMGA1 is expressed in this malignancy as well.

Materials and methods

Tissue samples

In the present study 170 breast carcinomas were analysed. For each tumour sample paraffin-embedded archival material was used. 51 samples were regular tissue sections and 119 breast cancer samples were used to prepare a tumour tissue array as previously described (Kononen et al., 1998). The spot diameter of the tissue array was 0.6 mm, and the distance between the spots was 1 mm. Histological subtypes were determined in accordance to Rosen and Oberman (1993) and tumour grading in accordance to Remmele (1997). Histological examination revealed the typical distribution of the common histological types of breast cancers with most samples being of the invasive ductal type (Table 1).

Immunohistochemistry

For immunohistochemical examination, 5μm sections of paraffin-embedded human breast cancer samples and of the tumour tissue array were used. Slides were pretreated in a pressure-cooker for 5 min in 0.02 M borate buffer (pH 7.0) and afterwards washed for three times for 5 min in Sörensen's phosphate buffer (0.15 M Na_2HPO_4, 0.15 M KH_2PO_4, pH 7.4). For immunohistochemical staining a rabbit polyclonal antibody raised against a recombinant protein correspondig to amino acids 1-95 representing full length HMGA1b of human origin (sc-8982, Santa Cruz Biotechnology, Santa Cruz, CA; 1:10) detecting both HMGA1a and HMGA1b was used. Immunohistochemistry was performed with an automated immunohistochemistry system, NexES IHC (Ventana Medical Systems, Strasbourg, France), using a labeled streptavidin biotin technique and an AEC (3-amino-9-ethylcarbazole) detection kit (Ventana Medical Systems). Bound primary antibodies were detected with a secondary biotinylated anti-rabbit IgG (Ventana Medical Systems) and a horse-radish peroxidase complex (Ventana Medical Systems). Tissue sections of human placenta known to express HMGA1 in the nuclei of the trophoblast served as positive controls for immunohistochemistry. Additionally, a negative control using phosphate buffer instead of the primary antibody was performed to exclude unspecific binding of the secondary antibody and immunoreaction of endogenous biotin and peroxidase. The negative control did not show any immunoreaction at all. Immunoreactivity was evaluated by an experienced pathologist by examination of staining intensity of the nuclei of tumour cells and percentage of positive cells using the immunoreactive score (IRS) according to Remmele and Stegner (1987). Immunohistochemistry was documented with a digital camera (AxioCam, Zeiss, Göttingen, Germany).

Results

We analysed 170 breast cancer samples by immunohistochemistry using an antibody raised against the human HMGA1a and HMGA1b proteins. As for the nuclei of cancer cells and corresponding stromal cells immunoreaction could be detected exclusively in the nuclei of breast cancer cells. Even at a first glance, a strong intertumoural heterogeneity of HMGA1 expression among the different samples, varying from samples without any positivity to tumours with a very strong immunoreaction, became apparent (Figs. 1, 2, Tables 1, 2). Based on the immunoreactive score (IRS) according to Remmele and Stegner (1987) 61 breast cancer samples were scored to IRS 0, eight to IRS 1, 21 to IRS 2, 14 to IRS 3, 19 to IRS 4, 23 to IRS 6, 12 to IRS 8, three to IRS 9, and nine to IRS 12 (Table 2). This means that 14.1% of the tumours showed a strong HMGA1 positivity (IRS 8-12), 24.7% showed a moderate positivity (IRS 4-6), 25.3% showed a weak

Table 1. Histology in accordance to Rosen and Oberman (1993) and immunoreactive score (IRS) of HMGA1 of 170 breast cancer samples analysed in this study.

HISTOLOGICAL TYPE OF BREAST CANCER	NUMBER OF TUMOURS AND IRS OF HMGA1								
	IRS 0	IRS 1	IRS 2	IRS 3	IRS 4	IRS 6	IRS 8	IRS 9	IRS 12
Invasive ductal carcinoma (n=101)	23	5	15	10	12	19	8	3	6
Invasive lobular carcinoma (n=25)	10	1	2	1	2	3	3	-	3
Medullary carcinoma (n=1)	-	-	1	-	-	-	-	-	-
Mucinous carcinoma (n=12)	7	2	-	-	2	1	-	-	-
Invasive papillary carcinoma (n=4)	4	-	-	-	-	-	-	-	-
Tubular carcinoma (n=26)	16	-	3	3	3	-	1	-	-
Invasive cribriform carcinoma (n=1)	1	-	-	-	-	-	-	-	-

positivity (IRS 1-3), and 35.9% of the tumours did not show any positivity for HMGA1 at all (IRS 0). Overall, 64.1% of all tumours analysed showed at least a weak HMGA1 positivity, i.e. 109 out of 170 invasive carcinomas. Statistical analysis using a Spearman-rang correlation test revealed a strong correlation between tumour grade and HMGA1 positivity ($r_s=0.3516$, $p<0.0001$) (Fig. 2). Immunoreaction could be detected in all histological types of breast cancers analysed with the exception of papillary and cribriform carcinoma (Table 1).

Discussion

The aim of this study was to analyse the expression patterns of HMGA1 in breast cancer samples. So far, HMGA1 expression has been studied in some larger series of human cancers including thyroid, prostatic, pancreatic, uterus cervical, and colorectal cancers. As for HMGA1 expression in breast cancer, all studies published so far have been performed on cell lines (Ram et al., 1993; Holth et al., 1997; Liu et al., 1999; Nacht et al., 1999; Banks et al., 2000; Scala et al., 2000; Dolde et

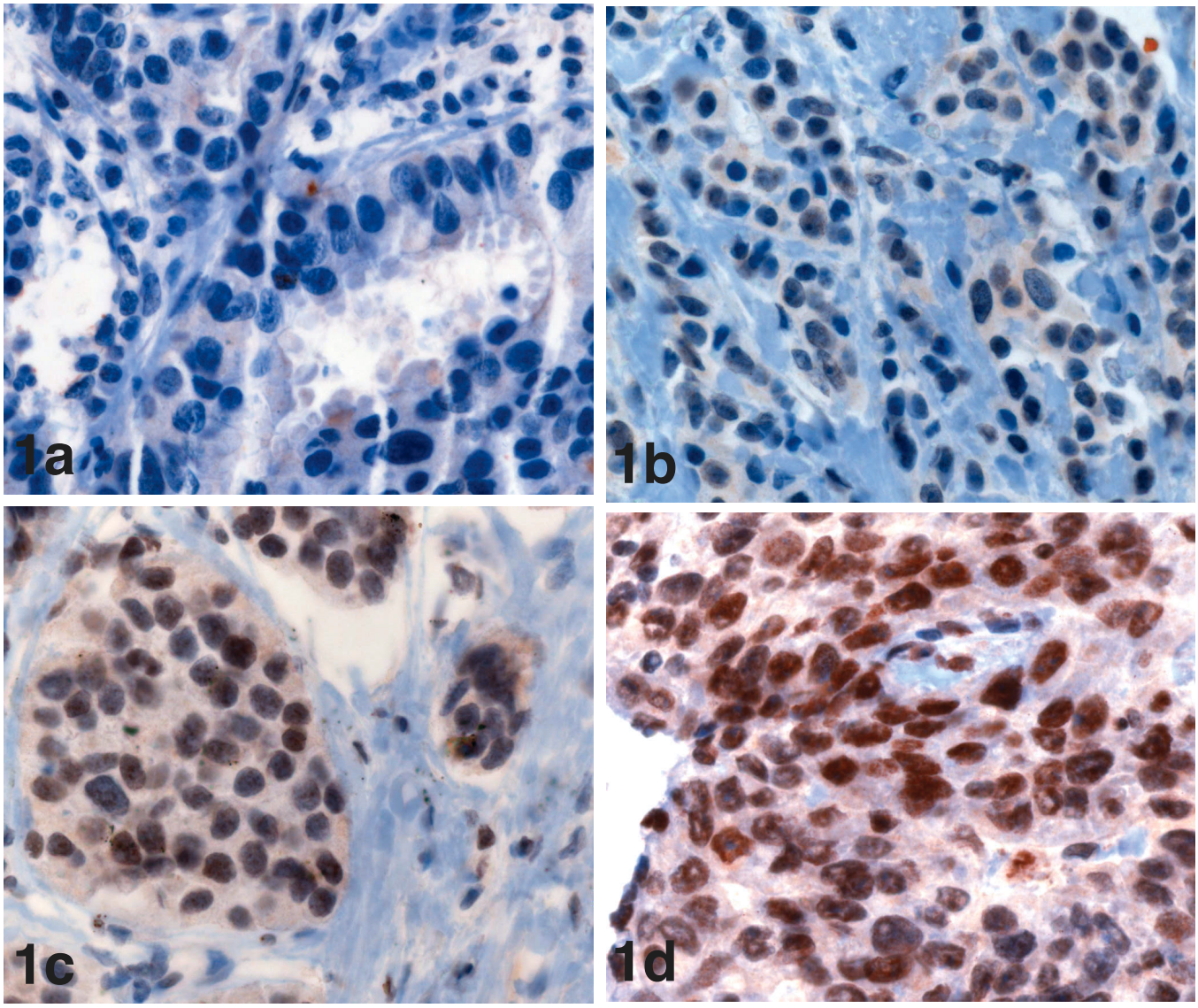

Fig. 1. Immunohistochemical analysis of four invasive breast cancer samples using an antibody raised against the human HMGA1a and HMGA1b proteins. Based on the immunoreactive score (IRS) according to Remmele and Stegner (1987) the four samples have been scored to IRS 0, 2, 6, and 12, respectively. HMGA1 positivity is detectable in the nuclei of breast cancer cells. **a.** Case 1: Invasive papillary carcinoma (grade 2, IRS for HMGA1: 0). **b**. Case 2: Invasive ductal carcinoma (grade 3, IRS for HMGA1: 2). **c.** Case 3: Invasive ductal carcinoma (grade 3, IRS for HMGA1: 6) **d.** Case 4: Invasive ductal carcinoma (grade 3, IRS for HMGA1: 12) x 400

al., 2002). However, the percentage of primary tumours affected by HMGA1 expression has not been described.

Herein, we show that HMGA1 protein expression varies strongly in a subset of 170 breast cancer samples revealing that 38.8% of the tumours were clearly positive (IRS 4-12), 25.3% had a weak HMGA1 positivity (IRS 1-3), and 35,9% did not show any positivity at all. These results are in good agreement with a study by Dolde et al. (2002) who found HMGA1 proteins increased in several human breast cancer cell lines compared to a cell line derived from normal breast cells. As for the role of HMGA1 in breast cancer Dolde et al. (2002) found that decreasing HMGA1a/b proteins inhibits transforming activity in soft agar of human breast cancer cells. Additionally, an increased expression of HMGA1a in normal human breast cells leads to neoplastic transformation (Dolde et al., 2002). Interestingly, based on our results approximately one third of the tumours do not express HMGA1, so that the protein is probably not absolutely necessary for the malignant transformation of human breast epithelium.

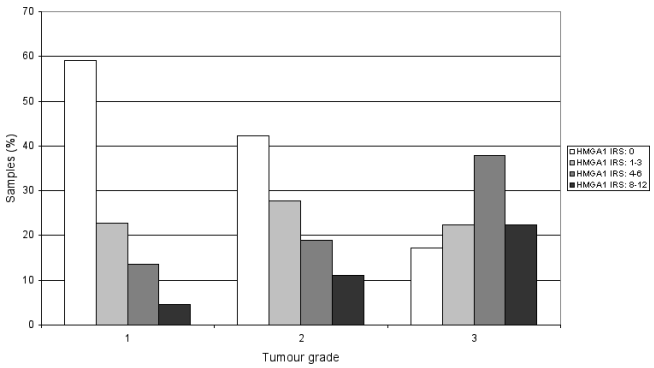

Fig. 2. Percentage of tumour samples with histological grade 1, 2, and 3, respectively, and different immunoreactive scores (IRS) of HMGA1 (HMGA1 IRS 0: no expression, HMGA1 IRS 1-3: weak expression, HMGA1 4-6: moderate expression, HMGA1 8-12: strong expression). Based on statistical analysis there is a strong positive correlation between HMGA1 expression and tumour grade.

Moreover, we found a strong positive correlation between histological grade and HMGA1 expression. This is in accordance with other studies showing a correlation between a malignant phenotype or high tumour grade and upregulation of HMGA1 (Tamimi et al., 1993; Chiappetta et al., 1995, 1998; Fedele et al., 1996; Bandiera et al., 1998; Abe et al., 1999, 2000). Accordingly, for these tumours an HMGA1 expression has been assumed to represent a prognostic factor.

As for therapeutic interventions aimed at an inhibition of HMGA1, Scala et al. (2000) were able to show that *in vivo* suppression of HMGA1 protein synthesis by an *HMGA1* antisense adenoviral vector may be a useful treatment strategy in a variety of human malignant neoplasias. Interestingly, this study includes breast cancer cell lines, i.e. MCF-7 and MDA 468, showing altered growth patterns depending on the infection by the antisense construct (Scala et al., 2000). However, because only a subgroup accounting for roughly two third of the breast cancer patients shows tumours expressing HMGA1, the proper determination of HMGA1 expression would be a prerequisite for all therapeutic approaches aimed at a suppression of the level of that protein.

This is the first report analysing HMGA1 expression patterns in primary breast cancer. HMGA1 positivity

Table 2. HMGA1 expression patterns obtained by immunohistochemistry and tumour grade of 170 breast cancer samples. For determination of nuclear HMGA1 positivity the immunoreactive score (IRS) according to Remmele and Stegner (1987) was used.

IRS FOR HMGA1	TUMOURS OF GRADE 1	TUMOURS OF GRADE 2	TUMOURS OF GRADE 3	NUMBERS OF TUMOURS WITH IRS
IRS 0	13	38	10	61 (35.9%)
IRS 1	0	4	4	8 (4.7%)
IRS 2	3	11	7	21 (12.4%)
IRS 3	2	10	2	14 (8.2%)
IRS 4	2	11	6	19 (11.2%)
IRS 6	1	6	16	23 (13.5%)
IRS 8	1	7	4	12 (7.1%)
IRS 9	0	1	2	3 (1.8%)
IRS 12	0	2	7	9 (5.3%)

was noted in approximately two thirds of breast cancer samples and a strong correlation of HMGA1 expression and tumour grade became apparent. These results suggest that HMGA1 expression can be considered a powerful prognostic marker for this malignancy. Moreover, determination of the HMGA1 expression level will be useful with regard to new therapeutic concepts aimed at the suppression of HMGA proteins (Scala et al., 2000).

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