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Detection of *HMGI-C* in the peripheral blood of breast cancer patients

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Abstract

The human high mobility group (HMG) protein (HMGI-C) belongs to the HMG family of architectural transcription factors which are expressed only during embryonic development, and not in normal adult tissues. Considerable interest has recently been shown in *HMGI-C* and its expression in a variety of neoplastic tissues, whereas no expression could be found in normal tissue adjacent to the tumour. So far, no data is available on the expression of *HMGI-C* in the peripheral blood of patients with solid tumours. In this study we analysed the expression of *HMGI-C* in peripheral blood samples of 61 patients with breast cancer and 35 healthy donors using a haemi-nested reverse transcriptase–polymerase chain reaction (RT-PCR) technique. No *HMGI-C* could be detected in any of the healthy donors' samples. In the three prognostic groups according to the Nottingham Prognostic Score, the proportion of patients expressing *HMGI-C* differed significantly ($P=0.001$). The worse the prognosis was, the more patients expressed *HMGI-C*. This is the first report on the expression of *HMGI-C* in the peripheral blood of patients with breast cancer and our data suggest that this expression is correlated with a poor prognosis.

Keywords: Breast cancer; High mobility group protein gene (*HMGI-C*); Prognosis

1. Introduction

HMG proteins are small non-histone chromosomal proteins, initially characterised by a high electrophoretic mobility in polyacrylamide gels (hence the acronym HMG) [1]. The HMGI family consists of three proteins: HMGI, HMGI(Y) and HMGI-C [1–4]. The former two are produced by differential splicing from a single gene and although the latter, HMGI-C, is encoded by a different gene, it shares some structural homologies with HMGI and HMGI(Y). HMGI-C has an approximately 50% amino acid sequence homology with HMGI(Y) [2]. All three proteins contain three DNA binding domains, short basic domains termed AT-hooks, which recognise AT-rich DNA sequences via the minor groove, and highly acidic C-terminal tails [1].

Recent studies have shown that they function as architectural transcription factors in the nuclear scaffold, with a role in the regulation of chromatin structure and function, being responsible for the correct three-dimensional configuration of protein–DNA complexes [2]. They promote gene activation during embryonal development and within rapidly dividing cells by facilitating enhanceosome formation on inducible genes via both protein/DNA and protein/protein interactions. HMGI-C was shown to enhance the activity of the transcription factor nuclear factor-kappa-B (NF-κB) [5]. The *HMGI-C* gene is normally exclusively expressed during embryonic development and in haematopoietic stem cells in adults, but it is undetectable in any other newborn and adult tissues by sensitive reverse transcriptase–polymerase chain reaction (RT-PCR) techniques [6]. Functionally knocking out the *HMGI-C* gene in mice leads to the pygmy phenotype, with a reduced birth weight and an adult body weight of approximately 40% of their wild-type littermates. This demonstrates the important role of *HMGI-C* in mammalian growth

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and development [7]. The importance of *HMGI-C* is also suggested by the fact that its genomic structure and chromosomal localisation has been conserved for at least 30 million years [8].

Direct evidence for *HMGI-C*'s participation in the oncogenic process was provided when the expression of antisense *HMGI-C* RNA was shown to prevent retrovirally induced neoplastic transformation in rat thyroid cells [9]. Considerable interest in the family member *HMGI-C* has been stimulated by observations that the gene, mapped to a 'multiple aberration region' (MAR) on chromosome 12, is re-expressed and/or rearranged in a number of tumours, namely in breast cancer, lung cancer, neuroblastoma, thyroid cancer, gastrointestinal cancer cell lines, leukaemia, uterine leiomyoma, endometrial polyps, pulmonary hamartoma, fibroadenoma of the breast, adenoma of the parotid glands, lipoma and sarcoma [10–16]. Intron 3 of *HMGI-C* can be considered the most frequent target of chromosomal aberrations in human tumours [8]. These findings suggest a pivotal role of *HMGI-C* in the control of cell growth, differentiation and tumorigenesis. Recent studies showed that while *HMGI-C* was expressed in the tumour tissue, it could not be detected in normal tissue adjacent to the tumour employing sensitive RT-PCR techniques and no *HMGI-C* could be found in peripheral blood samples of healthy donors with published methods [10,12]. As no data are available concerning the detection of *HMGI-C* in the peripheral blood of patients with solid tumours, we have analysed for the first time the *HMGI-C* expression in peripheral blood samples of 61 breast cancer patients in this study.

2. Patients and methods

Peripheral blood samples from 61 consecutive patients with breast cancer and 35 peripheral blood samples from healthy donors were analysed in the present study. Informed consent was given by patients and donors. Blood samples (5 ml) were immediately stabilised with DNA/RNA stabilisation reagent (Boehringer Mannheim, Germany) after being drawn from the patient or healthy donor and processed according to the manufacturer's instructions.

Briefly, mRNA was obtained using an mRNA kit (mRNA Isolation Kit, Boehringer Mannheim, Germany), cDNA was synthesised using the adapter primer (AP2) and Superscript II reverse transcriptase (Life Technologies, Eggenstein, Germany) and *HMGI-C* expression was determined using a hemi-nested RT–PCR as previously described [6]. The resulting PCR-product, a band of 220 base pairs (bp), was clearly visible after gel electrophoresis on a 2% agarose gel (Fig. 1). The resulting bands were sequenced and the sequence found to be identical with *HMGI-C* [17]. As a control reaction for intact RNA and cDNA, a PCR for the amplification of the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was performed for all samples to prevent false-negative PCR results. The PCR reaction was performed for 30 cycles under the same conditions as for the first round of the *HMGI-C* PCR. Only samples showing *GAPDH* expression were included in this study, three samples were excluded for this reason.

Statistical significance of the data was determined using the χ^2 test. A level of $P < 0.05$ was considered significant.

3. Results

All 35 peripheral blood samples from healthy blood donors were negative for *HMGI-C*. The 61 peripheral blood samples consisted of 20 samples from breast cancer patients without systemic metastases and 41 samples from patients with metastatic breast cancer. No *HMGI-C* could be detected in the blood samples from the former group. In the latter group 14/41 (34%) were *HMGI-C* positive and 27/41 (66%) were *HMGI-C* negative. As our results revealed a *HMGI-C* expression only in the blood of metastatic patients, we used the Nottingham Prognostic Score constructed for metastatic breast cancer to predict survival, to analyse the expression pattern of *HMGI-C* in our study [18]. This index, derived from a Cox model is scored (4×Grade)−(6×oestrogen receptor (ER))+(4×site of initial metastasis (SIMD))−(0.1×disease-free interval (DFI)), where histological grade is scored 1–3 (good, moderate or poor), ER is scored 0 (negative) or 1

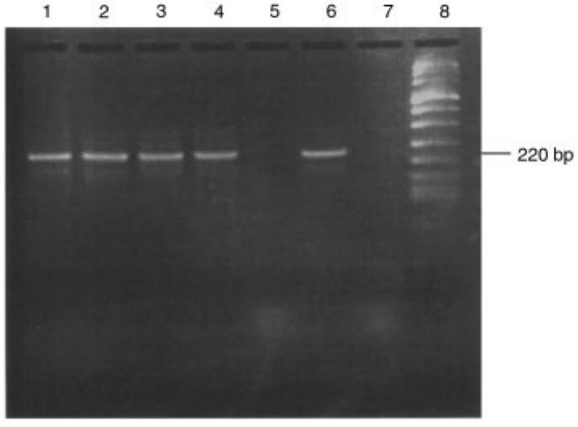

Fig. 1. Reverse transcriptase–polymerase chain reaction (RT-PCR) products of *HMGI-C* after gel electrophoresis and ethidium bromide staining, resulting in specific 220 bp bands, which were subsequently confirmed to be *HMGI-C* by sequencing analysis. Lanes 1–4, blood samples from metastatic breast cancer patients having a poor prognosis according to the Nottingham Prognostic Score; lane 5, healthy blood donor; lane 6, positive control (patient with myeloid leukaemia whose samples had previously been confirmed to express *HMGI-C*); lane 7, negative control (aqua bidest, starting at cDNA synthesis); lane 8, DNA molecular weight marker VIII (Boehringer Mannheim, Germany).

(positive), SIMD is scored 1–4 for bone only, lung only, bone and lung or visceral metastases, respectively, and DFI is measured in months. The DFI was defined as the time from 'potentially curative surgery' to the development of recurrent disease. Patients were divided into three prognostic groups on the basis of this score [18], group A <8.0, group B 8.0–16.5 and group C >16.5. The lower the index score was, the better the prognosis with a median survival of 26, 16 and 4 months being observed, respectively. The characteristics of patients with metastatic breast cancer, the respective Nottingham Prognostic Score and expression of *HMGI-C* in the peripheral blood are given in Table 1. In the three prognostic groups according to the Nottingham Prognostic Score, the proportion of patients expressing *HMGI-C* differed significantly ($P=0.001$). The worse the prognosis was, the more patients expressed *HMGI-C*. In group A, the group with the best prognosis, no (0/9) *HMGI-C* expression was detectable, in group B 3/15 (20%) of patients were *HMGI-C*-positive, whereas in group C, having the worst prognosis, 11/17 (65%) of the patients showed *HMGI-C* expression.

Table 1
Characteristics of patients with metastatic breast cancer, the respective Nottingham Prognostic Score and expression of *HMGI-C* in peripheral blood

Patients	Age (years)	Grade	ER	SIMD	DFI	Score	*HMGI-C*-positive
Prognostic group A = best prognosis							
1	66	2	1	Bone, thoracic wall	30	3	0
2	39	1	0	Bone, skin	36	4.4	0
3	63	2	1	Bone	0	6	0
4	61	1	1	Bone	36	<1	0
5	40	2	1	Bone, skin	0	6	0
6	68	No data	1	Lung, pleura	80	<8	0
7	73	2	1	Lung	42	5.8	0
8	65	2	1	Lung	48	5.2	0
9	65	2	No data	Lung	120	<8	0
Prognostic Group B = intermediate prognosis							
1	40	2	1	Lymph node, liver	60	12	0
2	38	2	1	Bone, lung	0	14	0
3	86	3	1	Bone	4	9.6	0
4	59	2	1	Bone, lung	40	10	1
5	59	1	1	Liver	36	10.4	1
6	69	2	No data	Bone, liver	96	<16.5	0
7	55	2	0	Bone	12	10.8	0
8	61	2	1	Bone, lung, liver	60	12	0
9	47	3	1	Lung	20	12	0
10	44	2	0	Lung, thoracic wall	18	14.2	0
11	70	3	1	Bone	0	10	0
12	57	2	0	Lung	70	9	0
13	37	2	1	Bone, lung	4	13.6	0
14	55	2	0	Lymph node, bone	24	9.6	1
15	59	3	0	Lung	38	16.2	0
Prognostic Group C = worst prognosis							
1	54	3	0	Lymph node, liver	12	26.8	1
2	60	3	0	Bone, lung, pleura	27	21.3	1
3	46	3	0	Liver	12	26.8	0
4	53	3	0	Lung, liver	12	26.8	1
5	64	2	1	Bone, bone marrow, lung	4	17.6	1
6	32	2	1	Lung, liver	10	17	0
7	49	3	1	Lymph node, liver	12	20.8	1
8	46	3	1	Lymph node, liver	12	20.8	1
9	52	3	No data	Lung, brain	0	>16.5	0
10	52	2	0	Bone, lung, liver	12	22.8	1
11	40	3	0	Lymph node, lung	24	17.6	1
12	51	2	0	Bone, lung, retina	0	20	0
13	41	3	0	Liver	0	28	0
14	46	3	1	Liver	0	22	1
15	65	2	0	Lung, liver	42	19.8	0
16	47	2	1	Liver	12	16.8	1
17	61	2	1	Bone, lung, liver, brain	12	16.8	1

ER, oestrogen receptor; SIMD, site of initial metastasis; DFI, disease-free interval (months); *HMGI-C*-positive, positive for expression of *HMGI-C* in peripheral blood.

4. Discussion

HMGI-C is an architectural transcription factor which is expressed in a variety of neoplastic tissues, but not in normal adult tissues. In a study on *HMGI-C* expression in breast cancer tissues, *HMGI-C* was predominantly noted in tumours with high histological grade, indicating a relationship between histological grade of the tumour and expression of *HMGI-C* [10]. So far, data on the expression of *HMGI-C* in peripheral blood is available for patients with leukaemia and healthy donors only, where *HMGI-C* expression was detected in the leukaemia patients but not in the healthy donors [12]. Expression patterns have not been investigated in patients with solid tumours.

Here, we report for the first time the expression of *HMGI-C* in the peripheral blood of breast cancer patients. In our study, *HMGI-C* was not detectable in any of the samples from healthy donors, whereas expression could be found in the peripheral blood of breast cancer patients. The expression was restricted to patients with metastatic disease. Our results suggest that the expression of *HMGI-C* is related to the prognosis of metastatic disease as indicated by the Nottingham Prognostic Score.

In neoplastic tissues, a correlation between *HMGI-C* expression and grading has been suggested. The appearance of a highly malignant phenotype in differentiated rat thyroid cells transformed with oncogenes has been demonstrated where *HMGI-C* expression is observed [9]. It was subsequently shown that inhibition of HMGI-C protein synthesis by an antisense methodology suppressed retrovirally induced neoplastic transformation of those thyroid cells. Similar observations were made for HMGI(Y). In a recent study on colorectal neoplastic tissues, a correlation could be found between an increased HMGI(Y) protein expression due to an increase in its mRNA and various clinicopathological parameters, known to be indicative of a poor prognosis [19]. These findings indicated that the determination of HMGI(Y) expression could be a potential prognostic factor for patients with colorectal cancer. A significant correlation between *HMGI(Y)* mRNA expression and tumour grade and stage was found in another study on prostate cancer [20]. These data indicate that HMG proteins play a crucial role in cancer and further research is needed to elucidate their particular role in tumour biology.

In this study, we show for the first time the expression of *HMGI-C* in the peripheral blood of patients with a solid tumour employing an RT–PCR technique and establish a relationship between its expression and prognosis. Further investigations are currently underway in our laboratory to characterise the impact of *HMGI-C* expression on the survival of patients with breast cancer and its role as a predictive factor.

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