High-mobility group A1 proteins are overexpressed in human leukaemias

Giovanna Maria PIERANTONI^{*1,2}, Valter AGOSTI^{†1}, Monica FEDELE^{*}, Heather BOND[‡], Irene CALIENDO[§], Gennaro CHIAPPETTA[¶], Francesco LO COCO^{||}, Fabrizio PANE[†], Maria Caterina TURCO[†], Giovanni MORRONE[‡], Salvatore VENUTA[‡] and Alfredo FUSCO^{*}

^{*}Dipartimento di Biologia e Patologia Cellulare e Molecolare, c/o Centro di Endocrinologia ed Oncologia Sperimentale del CNR, Facoltà di Medicina e Chirurgia, Università degli Studi di Napoli "Federico II", via S. Pansini, 5, 80131 Naples, Italy, [†]Dipartimento di Biochimica e Biotechnologie Mediche, Facoltà di Medicina e Chirurgia, Università degli Studi di Napoli "Federico II", via S. Pansini 5, 80131 Naples, Italy, [‡]Dipartimento di Medicina Sperimentale e Clinica, Facoltà di Medicina e Chirurgia, Università di Catanzaro, via Tommaso Campanella, 88100 Catanzaro, Italy, [§]A.O. San Giuseppe Moscati, viale Italia, 83100 Avellino, Italy, [¶]Istituto Nazionale dei Tumori di Napoli, Fondazione Senatore Pascale, via M. Semmola, 80131 Naples, Italy, and ^{||}Dipartimento di Biotechnologie Cellulari ed Ematologia, Università "La Sapienza", Rome, Italy

High-mobility group A (HMGA) proteins are non-histone nuclear proteins that bind DNA and several transcription factors. They are involved in the regulation of chromatin structure and function. HMGA protein expression is low in normal adult tissues, but abundant during embryonic development and in several human tumours. Rearrangements of the *HMGA* genes have been frequently detected in human benign tumours of mesenchymal origin, e.g. lipomas, lung hamartomas and uterine leiomyomas. HMGA proteins have been implicated in the control of cell growth and differentiation of the pre-adipocytic cell line 3T3-L1. In an attempt to better understand the role of HMGA1 proteins in haematological neoplasias and in the differentiation

of haematopoietic cells, we have investigated their expression in human leukaemias and in leukaemic cell lines induced to terminal differentiation. Here we report HMGA1 overexpression in most fresh human leukaemias of different origin and in several leukaemic cell lines. Moreover, differentiation of three cell lines towards the megakaryocytic phenotype was associated with HMGA1 protein induction, whereas induction of erythroid and monocytic differentiation generally resulted in reduced HMGA1 expression.

Key words: HL60 cells, *HMGA1*, K562 cells, leukaemia, megakaryocytic differentiation.

INTRODUCTION

The regulated expression of factors that modulate gene expression maintains the proliferation/differentiation balance in haematopoietic cells [1–3]. Studies of the expression and activity of these factors in human leukaemias may provide insights into the neoplastic processes in this compartment. The high-mobility group A (HMGA) family consists of three proteins: HMGA1a and HMGA1b, which result from alternative splicings of the same gene, i.e. *HMGA1* [4], and HMGA2, which is encoded by a different gene [5]. HMGA proteins are involved in the regulation of chromatin structure and function [6].

HMGA levels are high in embryonic tissues [7,8] and negligible in normal adult tissues. HMGA proteins have been implicated in the control of adipocytic homeostasis: mice with targeted disruption of *Hmga2* have severe deficiency of fat tissue [8], whereas suppression of HMGA1 protein synthesis in the pre-adipocytic 3T3-L1 cell line by antisense technology dramatically increased growth rate and impaired adipocytic differentiation [9].

Rearrangements of the *HMGA2* gene have been frequently found in human benign tumours, generally those of mesenchymal origin, e.g. lipomas, lung hamartomas and uterine leiomyomas [10,11]. They result from chromosomal aberrations of chromosomal region 12q13–15, which harbours the *HMGA2* gene. We previously reported a 12q13 translocation involving *HMGA2* in a case of Richter's transformation of a chronic lymphocytic leukaemia [12], and more recently we found a rearrangement of the *HMGA2* locus in an acute lymphocytic leukaemia (AML; G. M. Pierantoni, B. Santulli, I. Caliendo and A. Fusco, unpublished work).

HMGA proteins are overexpressed in several human malignant neoplasias including human thyroid [13,14], colon [15–17], prostate [18], cervical [19], ovarian (V. Masciullo and A. Fusco, unpublished work) and pancreatic [20] carcinomas. High levels of HMGA expression are causally related to a malignant phenotype. Indeed, unlike untransfected cells, thyroid cells transfected with an antisense construct for *Hmga2* or *Hmga1* cDNAs do not exhibit a malignant phenotype after infection with murine transforming retroviruses [21,22]. Moreover, HMGA1 protein overexpression is also required for cancer development in humans. In fact, an adenovirus carrying the *HMGA1* gene in an antisense orientation induced programmed cell death in cell lines derived from human thyroid, lung, colon and breast cancers [23].

In an attempt to understand the role of *HMGA1* in haematological neoplasias and in the differentiation of haematopoietic cells, we investigated HMGA1 protein expression in human leukaemias and in leukaemic cell lines induced to terminal differentiation. Here we report HMGA1 overexpression in most of the fresh human leukaemias and in leukaemic cell lines studied. Differentiation of the DAMI and K562 cell lines towards the megakaryocytic phenotype was associated with induction of the HMGA1 proteins, whereas induction of erythroid and monocytic differentiation generally resulted in reduced HMGA1 expression.

MATERIALS AND METHODS

Cells

The human promyelocytic HL60, the erythroleukaemic K562, the megakaryoblastic DAMI, the myeloid CD34+ KG-1 and

Abbreviations used: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HMGA, high-mobility group A; PBMNC, monocytes purified from peripheral blood of healthy volunteers; RT-PCR, reverse transcriptase PCR; AML, acute myeloblastic leukaemia.

¹ These authors contributed equally to this work.

² To whom correspondence should be addressed (e-mail gmpieran@unina.it).

the monocytoid U937 cell lines were obtained from the American Type Culture Collection (Manassas, VA, U.S.A.). The JURL-MK1 cell line was established from peripheral blood cells of a patient in a megakaryoblastic crisis phase of chronic myelogenous leukaemia [24]. All cells were grown in suspension culture in RPMI 1640 medium (Flow, Osaka, Japan) supplemented with 10 % fetal calf serum (Hyclone, Cramlington, Northumberland, U.K.), 100 units/ml of penicillin, 100 µg/ml streptomycin and 2 mM glutamine (Flow) in humidified 5 % CO₂. The human myeloid cell lines were induced to differentiate. The differentiation-inducing reagents, bovine haemin, PMA, ionomycin and sodium butyrate were from Sigma. To stimulate differentiation, KG-1 cells were cultured with 10⁻⁷ M PMA and 1.6 µg/ml ionomycin, HL60 cells were treated with 10⁻⁸ M PMA, DAMI cells were treated with 10⁻⁷ M PMA and K562 cells were treated with 10⁻⁸ M PMA, 1 mM sodium butyrate or 5 × 10⁻⁵ M bovine haemin. The expression of surface markers was measured by flow cytometry using FITC-labelled monoclonal antibodies (Becton Dickinson) and the cellular DNA content was monitored by flow cytometry after permeabilization of the cells and staining of the DNA with propidium iodide.

Monocytes were purified from peripheral blood of healthy volunteers (PBMNC) by adherence to plastic tissue-culture dishes (Falcon). To isolate normal granulocytes, PBMNC were incubated for 45 min at 37 °C with 1 % dextran to allow erythrocyte sedimentation, and the cells still in suspension were subjected to density gradient centrifugation (Ficoll-Hypaque, Amersham Biosciences; density = 1.077). The pellet contained almost exclusively mature granulocytes, as verified by Giemsa staining. Lymphocytes were isolated from peripheral blood by centrifugation at 400 g for 30–40 min on the Ficoll-Hypaque gradient, as indicated by manufacturer's protocol. The cells in interphase were collected and used for subsequent analysis when their purity was ≥ 90 %, as verified by flow cytometry analysis.

Primary peripheral blood leukaemic specimens were obtained from the Dipartimento di Biotecnologie Cellulari ed Ematologia, Università "La Sapienza", Rome, Italy, and from A.O. San Giuseppe Moscati, Avellino, Italy.

Northern-blot analysis

Total RNA was extracted by RNeasy (Tel-Test, Friendswood, TX, U.S.A.). Northern blots and hybridizations were carried out as described previously [25]. The *HMGA1* probe was derived from *pHMGA1* [4]. A 0.4 kb fragment corresponding to the cDNA of the human β-actin was used to check equal RNA loading. The hybridization signal was quantified with a Molecular Dynamics PhosphorImager. The images recorded by the PhosphorImager were analysed by volume integration with the ImageQuant software.

Reverse transcriptase PCR (RT-PCR) analysis of *HMGA1* expression

The RT-PCR procedure is described elsewhere [14]. Briefly, 1 µg of total RNA, digested with RNase-free DNase, was reverse-transcribed using random hexanucleotides as primers (100 mM) and 12 units of avian myeloblastosis virus reverse transcriptase (Gibco), and PCR amplification was performed as previously reported [26]. cDNA (200 ng) was amplified in a 25 µl reaction mixture containing *Taq* DNA polymerase buffer, 0.2 mM dNTPs, 1.5 mM MgCl₂, 0.4 mM of each

primer and 1 unit of *Taq* DNA polymerase (Perkin-Elmer). The PCR amplification was performed for 30 cycles (94 °C for 30 s, 55 °C for 1 min and 72 °C for 1 min). The sequences of primers used were: 5'-GGCACTGAGAAGCGGGGCCG-3' and 5'-CCCTTGTTTTTGCTTCCCTT-3' (corresponding to nucleotides 68–88 and nucleotides 161–141, respectively, of *HMGA1* cDNA). We added a set of primers specific for the constitutively expressed glyceraldehyde-3-phosphate dehydrogenase (GAPDH) to each reaction after 20 cycles of PCR as an internal control for the amount of cDNA tested. The GAPDH-specific primers were: forward primer, 5'-ACATGTTCCAATATGATTCC-3', corresponding to nucleotides 194–214; reverse primer, 5'-TGGACTCCACGACGTACTCAG-3', corresponding to nucleotides 356–336 of GAPDH cDNA. The products of the reaction were analysed on a 2 % agarose gel, and then transferred by electroblotting to GeneScreen plus nylon membrane (Dupont, Boston, MA, U.S.A.). The membranes were hybridized with a *HMGA1* cDNA probe. A PhosphorImager screen was briefly exposed to the membranes and the screen was scanned on a Molecular Dynamics PhosphorImager. The images recorded by the PhosphorImager were analysed by volume integration with ImageQuant software. The relative level of *HMGA1* gene expression was assessed by comparison with the level of GAPDH in the same sample.

Immunoblotting

Cells were scraped in PBS and lysed in Nonidet P40 lysis buffer supplemented with 50 mM NaF, 0.5 mM sodium vanadate, 0.5 mM PMSF, 5 mg/ml aprotinin and 5 mg/ml leupeptin. Proteins (50 µg) were separated on polyacrylamide gels and transferred on to nitrocellulose membranes (Hybond C; Amersham Biosciences). Membranes were blocked in 5 % non-fat dry milk, incubated with primary antibodies directed against the synthetic peptide SSSKQQPLASKQ specific for the HMGA1 proteins [13], detected with the appropriate secondary antibodies, and revealed by enhanced chemiluminescence (ECL; Amersham Biosciences). The antibodies against FRA-1 were from Santa Cruz Biotechnology. We used anti-γ-tubulin (Santa Cruz Biotechnology) to equalize the amounts of proteins loaded. In addition, membrane staining with Ponceau Red confirmed equal loading of protein lysates.

Immunohistochemical analysis of tissue samples

The patients' bone marrow smears were used for immunohistochemical studies. In brief, air-dried slides were washed in PBS, followed by quenching of endogenous peroxidase activity by 0.3 % H₂O₂ in methanol. After they were rinsed again with PBS, the smears were incubated with normal serum for 20 min at room temperature to block non-specific binding and then incubated with primary anti-HMGA1 antibody at a dilution of 1:75 for 14 h at 4 °C. After being washed in 0.2 % Triton-X100 in PBS, the sections were further incubated with biotinylated anti-rabbit IgG for 30 min (Dako LSAB Kit alkaline phosphatase; Dako Corporation) at room temperature, and then washed in 0.2 % Triton-X100 in PBS. After the addition of streptavidin-biotin conjugated to the alkaline phosphatase and incubation for 30 min at room temperature, the sections were washed in 0.2 % Triton-X100 in PBS, and the locations of the HMGA1 proteins were visualized by incubating the sections with fresh fuchsin chromogen. The slides were rinsed with PBS and mounted with a water-based mounting medium (Dako Glycergel).

Figure 1 Analysis of *HMGA1* gene expression in human leukaemic cell lines and leukaemia patients' cells

(A) RNA from human leukaemic cell lines was analysed by Northern blotting. Total RNA (5 μ g for each sample) was separated on a denaturing formaldehyde agarose gel, blotted on to nylon filters and probed with either *HMGA1* or β -actin cDNA, as indicated. Actin was used as an internal control for uniform RNA loading. Normal granulocytes were used as negative control. (B) Total RNA was extracted from leukaemia patients' cells and analysed by RT-PCR. Aliquots (1 μ g) of RNA were reverse transcribed, amplified as described in the Materials and methods section, then transferred to nitrocellulose and hybridized with *HMGA1* cDNA. GAPDH was used as an internal control for uniform RNA loading. Sources of RNA: M7, megakaryoblastic leukaemia; AML, acute myeloblastic leukaemia; ALL, acute lymphoblastic leukaemia; APL, acute promyelocytic leukaemia; CML, chronic myelogenous leukaemia. PBMNC were used as negative control.

RESULTS

HMGA1 is overexpressed in human leukaemic cell lines and in cells from leukaemia patients

We analysed *HMGA1* expression in six leukaemia cell lines (HL60, K562, KG-1, U937, DAMI and JURL-MK1) using Northern and Western blotting. *HMGA1* expression was high in all six cell lines, at both the mRNA (Figure 1A) and protein (results not shown) levels. Notably, the highest expression was observed in the DAMI and JURL-MK1 cell lines, which are of megakaryoblastic origin. Normal granulocytes did not show significant *HMGA1* expression (Figure 1A).

We also used RT-PCR to evaluate *HMGA1* expression in peripheral blood samples from 27 leukaemia patients: five megakaryoblastic leukaemias (M7), six AMLs of different Fab subtypes, six acute lymphoblastic leukaemias (ALLs), seven acute promyelocytic leukaemias (APLs) and three chronic myelogenous leukaemias (CMLs; see Figure 1B). *HMGA1* expression was high in all leukaemias, but undetectable in normal cells from PBMNC (Figure 1B).

We next analysed the bone marrow smears for *HMGA1* expression using immunohistochemistry and antibodies raised against the N-terminal region of the protein. Several leukaemia samples of diverse origin were analysed. In all cases analysed, the majority of blastic cells appear to be stained with anti-*HMGA1* antibody, showing a strong immunoreactivity that was not detectable in the normal bone marrow sample (results not shown). Representative results of the immunohistochemical analysis of two cases of AML are shown in Figures 2(C) and 2(D). Leukaemic cells of these samples can be distinguished by their blastic size in the Giemsa-stained samples, shown in Figures 2(A) and 2(B).

Cytoplasmic and perinuclear staining was observed only in leukaemic cells. The specificity of the reaction was verified by the

absence of immunoreactivity after pre-incubation of the antibody with a molar excess of the *HMGA1* recombinant protein (results not shown). Similarly, no signal was observed in the absence of the primary antibodies (results not shown).

HMGA1 expression in haematopoietic cell differentiation

To investigate whether *HMGA1* expression was related to haematopoietic differentiation, we treated K562 cells with PMA or haemin and sodium butyrate to induce megakaryocytic and erythroblastic differentiation, respectively, whereas KG-1 and HL60 cells were induced to monocytic differentiation by stimulation with PMA and ionomycin or PMA, respectively.

As shown in Figure 3(A), the megakaryocytic differentiation of K562 cells induced by PMA was accompanied by a remarkable increase in *HMGA1* mRNA levels. This increase was detected 6 h after stimulation with PMA and peaked at 18 h. Conversely, a slightly reduced *HMGA1* expression was observed when the same cells were driven towards erythroid differentiation by haemin and sodium butyrate (Figure 3B). Moreover, the induced differentiation of KG-1 and HL60 cells towards macrophages resulted in a drastic reduction of *HMGA1* gene expression (a 10-fold decrease as evaluated by densitometric analysis: Figures 3C and 3D, respectively). The results of Western-blot analysis of K562 and KG-1 cells after treatment with differentiating agents paralleled those obtained with Northern blotting (Figure 3E).

The effect of PMA on K562 and KG-1 cells was verified by a parallel analysis of FRA-1 protein, whose levels are known to be increased by this agent: FRA-1 protein was consistently increased in both K562 and KG-1 cells after incubation with PMA (Figure 4). Therefore, the differential expression of *HMGA1* in K562 and KG-1 cells by PMA was not due to a different activity of the agent in the two lines, but could rather be related to the

Figure 2 Immunohistochemical analysis of the HMGA1 expression in different leukaemia samples

(A and B) Giemsa staining of the bone marrow smear of two AML patients. Leukaemic cells can be distinguished by their blastic size. (C and D) Immunostaining of the bone marrow smear of the same patients (in A and B) by using antibodies against HMGA1 protein. Cytoplasmic and perinuclear staining was observed only in leukaemic cells.

differing haematopoietic differentiation of the two cell lines. To confirm the induction of differentiation, a variety of markers were used: in KG-1, a drastic decrease in the levels of CD34 mRNA was revealed by Northern blotting as described for monocytic differentiation [27,28]; the PMA-monocytic differentiation of HL60 was accompanied by a strong increase in the expression of the surface-membrane antigen CD11c, whereas the maturation of K562 and DAMI cells toward megakaryocytes were assessed by increased expression of CD61 and ploidy, respectively (results not shown). To verify the erythropoietic differentiation in sodium butyrate- and haemin-treated K562 cells, ζ -globin gene expression was evaluated by RT-PCR (results not shown).

The results shown above revealed an association between *HMGA1* gene induction and megakaryocytic differentiation. To confirm this association, we analysed *HMGA1* expression by Northern blotting in DAMI cells induced towards terminal megakaryocytic differentiation by PMA. As shown in Figure 5, *HMGA1* induction started at 6 h and increased further until 24 h of PMA treatment.

DISCUSSION

The HMGA proteins are chromatin proteins thought to be involved in tumorigenesis. Indeed they are overexpressed in all malignant tumours analysed so far [13–20] and rearranged in several human benign tumours of mesenchymal origin [10,11,29,30]. Here we show that HMGA1 overexpression is not restricted to solid malignant tumours, but is a common

feature of human leukaemias of diverse origin. Indeed, HMGA1 expression, which is not detectable in normal cells from peripheral blood of healthy volunteers, appears to correlate with the transformed state in these haematopoietic neoplasias. HMGA overexpression in human leukaemias probably plays an important role in the process of leukaemogenesis. In fact, HMGA proteins are required for neoplastic transformation, since the blockage of HMGA1 and HMGA2 protein synthesis prevents rat thyroid cell transformation by acute murine retroviruses [21,22]. Moreover, mice carrying a truncated *Hmga2* gene or wild-type *Hmga2* and *Hmgal* genes develop T-NK cell lymphomas [31,32] (for *Hmgal* results, M. Fedele, F. Pentimalli, G. Baldassarre, S. Battista, A.J.P. Klein-Szanto, L. Kenyon, C.M. Croce and A. Fusco, unpublished work). Furthermore, HMGA1 expression is increased in Burkitt's lymphoma cell lines, and rat 1a cells overexpressing HMGA1 protein form tumours with distant metastases in nude mice [33]. These data suggest that *HMGA* gene overexpression plays an important role in the generation of haematopoietic malignancies.

Therefore our findings provide an interesting perspective on gene therapy of human leukaemias based on the suppression of HMGA1 synthesis. In fact, HMGA1 protein overexpression is also required for expression of the malignant phenotype of human carcinoma cells, since an adenovirus carrying the *HMGA1* gene in an antisense orientation induced cell death in thyroid carcinoma cell lines [23].

In addition to its relation with a transformed state, HMGA1 expression could be a feature of normal megakaryocytic

Figure 3 Analysis of HMGA1 expression in haematopoietic cell differentiation

Total RNA from K562 (A and B), KG-1 (C) and HL60 (D) cells treated with PMA (TPA; and ionomycin for KG-1 cells) or with haemin and sodium butyrate (for K562 cells) for different periods of stimulation was analysed by Northern blotting. Actin was used as an internal control for uniform RNA loading. (E) Total proteins extracted from non-treated (–) and PMA-treated K562 and KG-1 cells were separated (50 μ g/lane) on SDS/PAGE and transferred to nitrocellulose membranes. Western blots were first incubated with antibodies specific for HMGA1 protein, and then with horseradish peroxidase-conjugated secondary antibodies; the immunocomplexes were detected by enhanced chemiluminescence. As a control for equal loading, the blotted proteins were stained with Ponceau Red. Moreover, the same Western blots were incubated with antibodies versus the ubiquitous γ -tubulin protein. Proteins were extracted from the cells at time 0, 6 and 18 h of treatment as indicated.

differentiation. Indeed, HMGA1 mRNA and protein levels were increased in K562 and DAMI cells driven towards the megakaryocytic phenotype by PMA induction [34,35], whereas the inducer had an opposite effect on KG-1 or HL60 cells, which differentiated towards macrophages [36]. Conversely, FRA-1 protein, whose levels are known to be increased by this agent, is induced after PMA treatment of K562 and KG-1 cells. These observations suggest that induction of the *HMGA1* gene may be related to megakaryocytic differentiation. Preliminary data from our laboratory seem to support this hypothesis: *HMGA1*^{–/–} embryonic stem cells induced to differentiate towards the megakaryocyte lineage by retinoic acid showed an increase of megakaryocyte proliferation and overexpression of early

Figure 4 Analysis of FRA-1 expression in PMA-stimulated cells

Western blot analysis of total proteins (50 μ g/lane) extracted from untreated and PMA (TPA)-treated K562 and KG-1 cells at time 0, 6 and 18 h of treatment. The membranes were first incubated with antibodies specific for FRA-1 protein, and then with the anti- γ -tubulin protein as a control for protein loading.

Figure 5 Analysis of HMGA1 expression during megakaryoblastic differentiation

Total RNA (20 μ g/lane) extracted from untreated and PMA-treated DAMI cells was analysed by Northern blotting for HMGA1 expression. The same filter was subsequently hybridized with a β -actin probe as a control for RNA loading.

megakaryocyte markers (S. Battista, F. Pentimalli, M. Fedele, G. Baldassarre, C. M. Croce and A. Fusco, unpublished work), a phenotype often associated with a maturation defect [37].

In addition, the reduction of HMGA1 expression in haemin- and sodium butyrate-treated K562 cells may be important in the process of erythropoiesis, and this is consistent with the increased ability of the *HMGA1*^{–/–} embryonic stem cells to give rise to erythroid colonies in methylcellulose (S. Battista, F. Pentimalli, M. Fedele, G. Baldassarre, C. M. Croce and A. Fusco, unpublished work).

In conclusion, our findings indicate that *HMGA1* gene overexpression is a common feature in leukaemias. Therefore, suppression of HMGA1 protein synthesis may be a useful treatment strategy in human leukaemias. Moreover, our data suggest that HMGA1 protein induction plays a functional role in megakaryocytic differentiation, whereas its reduction may be required for erythroid or macrophage differentiation.

This work has been supported by grants from AIRC (Progetto Speciale Oncosoppressori), the Progetto Finalizzato "Biotecnologie" of the CNR, the MURST projects "Terapie antineoplastiche innovative" and "Piani di Potenziamento della Rete Scientifica e Tecnologica", and from the Ministero della Sanità. We thank the Associazione Partenopea per le Ricerche Oncologiche (APRO) for its support. We are grateful to Jean Ann Gilder (Scientific Communication, Naples, Italy) for editing the text prior to submission.

REFERENCES

- Cantor, A. B. and Orkin, S. H. (2001) Hematopoietic development: a balancing act. *Curr. Opin. Genet. Dev.* **11**, 513–519.
- Petrella, A., Doti, I., Agosti, V., Giarrusso, P. C., Vitale, D., Bond, H. M., Cuomo, C., Tassone, P., Franco, B., Ballabio, A. et al. (1998) A 5' regulatory sequence containing two Ets motifs controls the expression of the Wiskott-Aldrich syndrome protein (WASP) gene in human hematopoietic cells. *Blood* **91**, 4554–4560.
- Romano, M. F., Lamberti, A., Bisogni, R., Garbi, C., Pagnano, A. M., Auletta, P., Tassone, P., Turco, M. C. and Venuta, S. (1999) Amifostine inhibits hematopoietic progenitor cell apoptosis by activating NF- κ B/Rel transcription factors. *Blood* **94**, 4060–4066.
- Johnson, K. R., Lehn, D. A. and Reeves, R. (1989) Alternative processing of mRNA's coding for human HMGI and HMGI(Y) proteins. *Mol. Cell. Biol.* **9**, 2114–2123.
- Manfioletti, G., Giancotti, V., Bandiera, A., Buratti, E., Sautiwe, P., Cary, P., Crane-Robinson, C., Coles, B. and Goodwin, G. H. (1991) cDNA cloning of the HMGI-C phosphoprotein, a nuclear protein associated with neoplastic and undifferentiated phenotypes. *Nucleic Acids Res.* **19**, 6793–6797.
- Lovell-Badge, R. (1995) Developmental genetics. Living with bad architecture. *Nature (London)* **376**, 725–726.
- Chiappetta, G., Avantaggiato, V., Visconti, R., Fedele, M., Battista, S., Trapasso, F., Merciai, B., Fidanza, V., Giancotti, V., Santoro, M. et al. (1996) High level expression of the HMGI(Y) gene during embryonic development. *Oncogene* **13**, 2439–2446.
- Zhou, X., Benson, K. F., Ashar, H. R. and Chada, K. (1995) Mutation responsible for the mouse pygmy phenotype in the developmentally regulated factors HMGI-C. *Nature (London)* **377**, 771–774.
- Melillo, R. M., Pierantoni, G. M., Scala, S., Battista, S., Fedele, M., Stella, A., De Biasio, M. C., Chiappetta, G., Fidanza, V., Condorelli, G. et al. (2001) Critical role of the HMGI(Y) proteins in adipocytic cell growth and differentiation. *Mol. Cell. Biol.* **21**, 2485–2495.
- Ashar, H. R., Schoenberg Fejzo, M., Tkachenko, A., Zhou, X., Fletcher, J. A., Weremowicz, S., Morton, C. C. and Chada, K. (1995) Disruption of the architectural factor HMGI-C: DNA-binding AT hook motifs fused in lipomas to distinct transcriptional regulatory domains. *Cell* **82**, 57–65.
- Schoenmakers, E. F., Wanschura, S., Mols, R., Bullerdiek, J., Van den Berghe, H. and Van de Ven, W. J. M. (1995) Recurrent rearrangements in the high mobility group gene HMGI-C: DNA-binding AT hook motifs fused in lipomas to distinct transcriptional regulatory domains. *Cell* **82**, 57–65.
- Santulli, B., Kazmierczak, B., Napolitano, R., Caliendo, I., Chiappetta, G., Rippe, V., Bullerdiek, J. and Fusco, A. (2000) A 12q13 translocation involving the HMGI-C gene in Richter transformation of a chronic lymphocytic leukemia. *Cancer Genet. Cytogenet.* **119**, 70–73.
- Chiappetta, G., Bandiera, A., Berlingieri, M. T., Visconti, R., Manfioletti, G., Battista, S., Martinez-Tello, F. J., Santoro, M., Giancotti, V. and Fusco, A. (1995) The expression of the high mobility group HMGI(Y) proteins correlates with malignant phenotype of human thyroid neoplasias. *Oncogene* **10**, 1307–1314.
- Chiappetta, G., Tallini, G., De Biasio, M. C., Manfioletti, G., Martinez-Tello, F. J., Pentimalli, F., de Nigris, F., Mastro, A., Botti, G., Fedele, M. et al. (1998) Detection of high mobility group I HMGI(Y) proteins in the diagnosis of thyroid tumors: HMGI(Y) expression represents a potential diagnostic indicator of carcinoma. *Cancer Res.* **58**, 4193–4198.
- Fedele, M., Bandiera, A., Chiappetta, G., Battista, S., Viglietto, G., Manfioletti, G., Casamassimi, A., Santoro, M., Giancotti, V. and Fusco, A. (1996) Human colorectal carcinomas express high levels of high mobility group HMGI(Y) proteins. *Cancer Res.* **56**, 1896–1901.
- Abe, N., Watanabe, T., Sugiyama, M., Uchimura, H., Chiappetta, G., Fusco, A. and Atomi, Y. (1999) Determination of high mobility group I(Y) expression level in colorectal neoplasias: a potential diagnostic marker. *Cancer Res.* **59**, 1169–1174.
- Chiappetta, G., Manfioletti, G., Pentimalli, F., Abe, N., Di Bonito, M., Vento, M. T., Giuliano, A., Fedele, M., Viglietto, G., Santoro, M. et al. (2001) High mobility group HMGI(Y) protein expression in human colorectal hyperplastic and neoplastic diseases. *Int. J. Cancer* **91**, 147–151.
- Tamimi, Y., van der Poel, H. G., Denyn, M. M., Umbas, R., Karthaus, H. F. M., Debruyne, F. M. J. and Shalcken, J. A. (1993) Increased expression of high mobility group proteins I(Y) in high-grade prostate cancer determined by *in situ* hybridization. *Cancer Res.* **53**, 5512–5516.
- Bandiera, A., Bonifacio, D., Manfioletti, G., Mantovani, F., Rustighi, A., Zancanati, F., Fusco, A., Bonito, L. D. and Giancotti, V. (1998) Expression of HMGI(Y) proteins in squamous intraepithelial and invasive lesions of the uterine cervix. *Cancer Res.* **58**, 426–431.
- Abe, N., Watanabe, T., Masaki, T., Mori, T., Sugiyama, M., Uchimura, H., Fujioka, Y., Chiappetta, G., Fusco, A. and Atomi, Y. (2000) Pancreatic duct cell carcinomas express high levels of high mobility group I(Y) proteins. *Cancer Res.* **60**, 3117–3122.
- Berlingieri, M. T., Manfioletti, G., Santoro, M., Bandiera, A., Visconti, R., Giancotti, V. and Fusco, A. (1995) Inhibition of HMGI-C protein synthesis suppresses retrovirally induced neoplastic transformation of rat thyroid cells. *Mol. Cell. Biol.* **15**, 1545–1553.
- Berlingieri, M. T., Pierantoni, G. M., Giancotti, V., Santoro, M. and Fusco, A. (2002) Thyroid cell transformation requires the expression of the HMGA1 proteins. *Oncogene* **21**, 2971–2980.
- Scala, S., Portella, G., Fedele, M., Chiappetta, G. and Fusco, A. (2000) Adenovirus-mediated suppression of HMGI(Y) protein synthesis as potential therapy of human malignant neoplasias. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 4256–4261.
- Di Noto, R., Luciano, L., Lo Pardo, C., Ferrara, F., Frigeri, F., Mercurio, O., Lombardi, M. L., Pane, F., Vacca, C., Manzo, C. et al. (1997) JURL-MK1 (c-kit(high)/CD30-/CD40-) and JURL-MK2 (c-kit(low)/CD30+/CD40+) cell lines: 'two-sided' model for investigating leukemic megakaryocytopoiesis. *Leukemia* **11**, 1554–1564.
- Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989) *Molecular Cloning: a Laboratory Manual*, 2nd edn, Cold Spring Harbor Press, Cold Spring Harbor, NY.
- Jackson, D. P., Quirke, P., Lewis, F., Boylston, A. W., Sloan, J. M., Robertson, D. and Taylor, G. R. (1989) Detection of measles virus RNA in paraffin-embedded tissue. *Lancet* **1**, 1391.
- Morrone, G., Bond, H. M., Cuomo, C., Agosti, V., Petrella, A., Pagnano, A. M., Della Corte, A., Marasco, O. and Venuta, S. (1995) Differential regulation of the expression of interleukin 2 receptor γ chain during the *in vitro* differentiation of human myeloid cells. *Biochem. J.* **308**, 909–914.
- Bond, H. M., Bonelli, P., Mesuraca, M., Agosti, V., Masone, C., Cuomo, C., Nistico, A., Tassone, P., Tuccillo, F., Cecco, L. et al. (1998) Identification by differential display of transcripts regulated during hematopoietic differentiation. *Stem Cells* **16**, 136–143.
- Tkachenko, A., Ashar, H. R., Meloni, A. R., Sandberg, A. and Chada, K. (1997) Misexpression of disrupted HMGI architectural factors activates alternative pathways of tumorigenesis. *Cancer Res.* **57**, 2276–2280.
- Xiao, S., Lux, M. L., Reeves, R., Hudson, T. J. and Fletcher, J. A. (1997) HMGI(Y) activation by chromosome 6p21 rearrangements in multilineage mesenchymal cells from pulmonary hamartoma. *Am. J. Pathol.* **150**, 901–910.
- Baldassarre, G., Fedele, M., Battista, S., Vecchione, A., Klein-Szanto, A. J., Santoro, M., Waldmann, T. A., Azimi, N., Croce, C. M. and Fusco, A. (2001) Onset of natural killer cell lymphomas in transgenic mice carrying a truncated HMGI-C gene by the chronic stimulation of the IL-2 and IL-15 pathway. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 7970–7975.
- Fedele, M., Battista, S., Kenyon, L., Baldassarre, G., Fidanza, V., Klein-Szanto, A. J., Parlow, A. F., Visone, R., Pierantoni, G. M., Outwater, E. et al. (2002) Overexpression of the HMGA2 gene in transgenic mice leads to the onset of pituitary adenomas. *Oncogene* **21**, 3190–3198.
- Wood, L. J., Mukherjee, M., Dolde, C. E., Xu, Y., Maher, J. F., Bunton, T. E., Williams, J. B. and Resar, L. M. (2000) HMGI(Y), a new c-Myc target gene and potential oncogene. *Mol. Cell. Biol.* **20**, 5490–5502.
- Nakajima, O., Iwasaki, S. and Hashimoto, Y. (1997) Hemin-induced erythroid differentiation of human myeloid leukemia K562 cell line and its modification by bioresponse modifiers. *Cell. Mol. Biol.* **43**, 115–134.
- Tetteroo, P. A., Massaro, F., Mulder, A., Schreuder-van Gelder, R. and von dem Borne, A. E. (1984) Megakaryoblastic differentiation of proerythroblastic K562 cell-line cells. *Leuk. Res.* **8**, 197–206.
- Koeffler, H. P., Bar-Eli, M. and Territo, M. C. (1981) Phorbol ester effect on differentiation of human myeloid leukemia cell lines blocked at different stages of maturation. *Cancer Res.* **41**, 919–926.
- Shivdasani, R. A., Rosenblatt, M. F., Zucker-Franklin, D., Jackson, C. W., Hunt, P., Saris, C. J. and Orkin, S. H. (1995) Transcription factor NF-E2 is required for platelet formation independent of the actions of thrombopoietin/MGDF in megakaryocyte development. *Cell* **81**, 695–704.