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HMGA1 protein is a positive regulator of the insulin-like growth factor-I receptor gene

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ABSTRACT

The IGF-I receptor (IGF-IR) is often overexpressed in cancer and is believed to play a crucial role in cancer progression. High Mobility Group A1 (HMGA1) is a non-histone chromatin protein that has the ability to regulate gene expression through DNA binding and involvement in enhanceosome complexes. HMGA1 is expressed at low level in adult differentiated cells, whereas it is expressed at high level in embryonic and malignant cells.

We evaluated whether the HMGA1 aberrant expression has a role in IGF-IR overexpression in cancer. We found that HMGA1 silencing induces a marked decrease in IGF-IR expression in various human cancer cell lines. Conversely, forced HMGA1 overexpression in cells with low endogenous HMGA1 levels was associated with IGF-IR upregulation. HMGA1 silencing reduced *igf-ir* promoter activity whereas forced HMGA1 expression increased it. Using the chromatin immunoprecipitation assay, HMGA1 protein was found to bind to the *igf-ir* promoter. Moreover, HMGA1 was found to associate with both p53 and Sp1, two major regulators of *igf-ir* gene transcription and to antagonise the p53 inhibitory activity while enhancing the Sp1 stimulatory activity.

Our data indicate, therefore, that HMGA1 protein is a positive regulator of IGF-IR expression and that HMGA1 overexpression may contribute to IGF-IR dysregulation in cancer cells.

1. Introduction

Various lines of evidence indicate that the insulin-like growth factor-I receptor (IGF-IR) plays a crucial role both in the regulation of normal growth and development and in carcinogenesis.[1] The IGF-IR role in development is exemplified by studies carried out in transgenic *igf-ir*$^{-/-}$ mice, which show a markedly reduced size at birth and die soon afterwards.[2] Moreover, the IGF-IR plays a permissive role on cell transformation: *igf-ir* knock-out mouse fibroblasts become unable to undergo transformation by several oncogenes.[3] In these fibroblasts IGF-IR re-expression not only restores the ability to undergo transformation in response to oncogenes but also induces ligand-dependent transformation if IGF-IR level exceeds a threshold value.[4] In agreement with these findings, malignant cells often overexpress the IGF-IR.[5] Finally, epidemiological

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studies indicate that circulating levels of bioactive IGF-I above normal levels or just at the upper quartile of the normal range are associated with an increased risk of developing various common malignancies.[6] Taken together, these studies indicate that the IGF-IR plays a crucial role in cancer biology[5] and justify the fact that the IGF-IR has become an important target for anticancer therapies.[7,8]

The mechanisms causing deregulated IGF-IR expression in cancer are incompletely understood.[9,10] In both physiological and pathological conditions the level of IGF-IR expression is primarily determined at the transcriptional level.[9] The *igf-ir* promoter region belongs to the class of TATA-less promoters and contains multiple GC boxes, potential binding sites for Sp1 and a transcription factor involved in *igf-ir* promoter constitutive activity. In addition, the promoter region includes binding sequences for the Wilms' tumour suppressor, WT1.[11] Finally, nuclear factors, such as p53 and Breast Cancer 1 (BRCA1), negatively regulate *igf-ir* transcription, in part through a functional interaction with Sp1.[11,12] Oncogenic counterparts of these nuclear factors may represent possible mechanisms for IGF-IR dysregulation in cancer.[9]

The High Mobility Group A (HMGA, i.e. HMGA1 and HMGA2) proteins are non-histone chromatin proteins that function as both positive and negative regulators of gene transcription.[13] These proteins may regulate gene transcription not only by direct binding to the minor groove of DNA and affecting DNA structure but also by binding to a variety of transcription factors and participating to the formation of multiprotein enhanceosome complexes.[13,14] Several lines of evidence led us to postulate that the HMGA proteins could be involved in *igf-ir* regulation. HMGA proteins are expressed at low level in differentiated adult cells but at very high level in embryonic and cancer cells,[13–15] and have been causally related to cell growth and to the malignant/metastatic phenotype. HMGA expression increases when cells are induced to proliferate.[13] In MCF-7 breast cancer cells forced overexpression of HMGA1 induced a gene expression programme compatible with dedifferentiation and epithelial-mesenchymal transition,[16] while HMGA1 silencing reverted the malignant phenotype in thyroid cancer cells.[17] Finally, HMGA1 has been implicated in the regulation of the insulin receptor gene (*ir*),[18] which is closely related to the *igf-ir* gene, although subjected to different regulation and expression patterns.[19–21]

In the present study we show that HMGA1 protein is a positive regulator of *igf-ir* gene expression and that it may contribute to IGF-IR overexpression in cancer cells.

2. Materials and methods

2.1. Cell cultures

The human hepatoblastoma cells HepG2 and the human embryonic kidney cells, HEK293 and HEK293T, were from ATCC (Manassas, VA). HepG2 were grown in MEM, while HEK293 and HEK293T were grown in Dulbecco's modified Eagle's medium (DMEM). Papillary thyroid cancer cells TPC-1 were provided by Drs. A. Fusco and M. Santoro (Naples, Italy) and anaplastic thyroid cancer cells SW1736 were provided by Dr. N.E. Heldin (Uppsala, Sweden). Both these cell lines were grown in RPMI 1640. The human osteosarcoma cell line Saos2 was provided by Dr. J.Y. Wang (La Jolla, CA) and was cultured in DMEM. Media were supplemented with 10% (v/v) FBS.

2.2. Gene silencing by siRNA

Cells plated onto six-well plates were transiently transfected with either scramble small interfering RNA (siRNA) or a mixture of four HMGA1 siRNAs at 100 nM final concentration (Dharmacon Research, Inc., Lafayette, CO), using Lipofectamine 2000 (Invitrogen, Pasley, UK). Forty-eight hours after transfection, the cells were lysated and analysed by Western blot.

2.3. Western blotting analysis

Cell lysates prepared from either untransfected or transiently transfected cells were subjected to SDS–PAGE. The resolved proteins were transferred to nitrocellulose membranes and were subjected to Western blot. Results were revealed by the ECL method (Amersham, Little Chalfont, UK), and analysed by densitometry. The following antibodies were used: anti-p53 monoclonal antibody DO-1, anti-Sp1 polyclonal antibody and anti-IGF-IR β-subunit polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA), anti-IR β-subunit polyclonal antibody (Transduction Laboratories, Lexington, KY, USA) and anti-β-actin antibody (Sigma, St. Louis, MO). Anti-HMGA1 polyclonal antibodies were obtained as previously described.[22]

For immunoprecipitation experiments, 1 mg of cell lysate was incubated for 2 h with 2 μg of antibody and protein G-Sepharose (GE Healthcare, Buckinghamshire, UK). Anti-p53 antibody DO-1, anti-Sp1 and anti-HMGA1 antibodies were used for immunoprecipitation.

2.4. Transient transfection and reporter assays

To assess the effect of HMGA1 on *igf-ir* promoter activity, HEK293 and Saos2 cells were transiently transfected with a HA-HMGA1 construct (in pcDNA3), as previously described.[23] The concomitant effect of p53 and Sp1 was studied by transfecting cells with constructs encoding for the human p53 (HA-pcDNA-p53) and human Sp1. We used two different *igf-ir* promoter reporters: one including 476 bp of 5′-flanking and 640 bp of 5′-untranslated regions of the *igf-ir* gene [p(–476/+640) luciferase] (representing the proximal *igf-ir* promoter) and a second one containing a minimal *igf-ir* promoter fragment, p(–40/+640) Luc.[24]

Cells seeded in 24-well plates were cotransfected with 0.3 μg of the *igf-ir* promoter reporter along with 0.2 μg of the HA-HMGA1 expression vector (or empty HA-pcDNA3) unless otherwise indicated, and with 0.2 μg of the p53 and Sp1 expression plasmids. The cells were lysed and processed according to the manufacturer's instructions (Promega Corp., Madison, WI). Luciferase activity was normalised for transfection efficiency using a vector coding for the Renilla gene.

2.5. Chromatin immunoprecipitation (ChIP) studies

HEK293T cells were transfected with a HMGA1 expression vector (HA-HMGA1, 6 μg) (or with empty HA-pcDNA3 vector, for control purposes), using the Jet-PEI transfection reagent

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(Polyplus, Illkirch, France). Forty-eight hours after transfection the cells were incubated with formaldehyde (1% final concentration) for 10 min at room temperature. At the end of the incubation period, the cells were washed twice and harvested using ice-cold phosphate-buffered saline. Pelleted cells were resuspended in 1% SDS (w/v) containing buffer, incubated on ice for 10 min and sonicated for 6 min (pulse time: 30 s and rest time: 30 s). The cell extracts were then immunoprecipitated with anti-HA for 18 h at 4 °C. For PCR analysis of HA-immunoprecipitated chromatin, a set of primers encompassing the IGF-IR proximal promoter region (nt –486 to +287) was employed: sense, CCAGCCGCGCTGTTGTTG and anti-sense, GGCTCGCTGAAGGTCACAG. PCR was performed using the TermalAce™ DNA polymerase reagent (Invitrogen). As a positive control (input DNA), the PCR was performed using lysates of transfected cells that were not immunoprecipitated with HA. As a negative control, the PCR was performed in the absence of DNA.

2.6. *Cell growth studies*

TPC-1 cells transfected with siRNAs against HMGA1 or scramble siRNAs or untransfected were seeded in 96-well plates. After 12 h of serum starvation, insulin or IGF-I (10 nM) was added. Cell growth was measured after 48 h by the MTT assay (Amersham Pharmacia Biotech). Cell proliferation was also evaluated using a time-resolved fluorometric immunoassay based on the incorporation of 5-bromo-2-deoxyuridine (BrdU) into newly synthesised DNA. BrdU (10 μM) was added during the last 6 h of ligand incubation. Incorporated BrdU was detected using europium-labelled monoclonal antibody and fluorescence activity was measured by the multilabel counter Wallac 1420 VICTOR3 (Perkin–Elmer).

2.7. *Statistical analysis*

Differences between means were analysed by the Student's *t*-test for paired samples. A *P*-value < 0.05 was considered statistically significant. Statistical analysis was performed with GraphPad Prism 4 software (GraphPad Software, Inc., La Jolla, CA).

3. Results

3.1. *HMGA1 regulates IGF-IR expression in cultured human cancer cells*

To avoid the possible interference of mutated p53 which might cause IGF-IR overexpression in cancer cells, we first studied HMGA1 silencing in HepG2 and TPC-1 cells, both expressing wild-type p53 and characterised by relatively high IGF-IR and IR content[25,26] and by the ability to respond to IGF-I with biological effects.

HepG2 and TPC-1 cells were transfected either with specific siRNAs against HMGA1 or with scramble control siRNA. The expression of HMGA1, IGF-IR and IR proteins was then evaluated in transfected cells by immunoblotting. In both HepG2 and TPC-1, cell transfection with HMGA1 siRNAs induced a marked reduction of HMGA1 protein (to 28 ± 4% and 33 ± 6% of control values, in HepG2 and TPC-1, respectively) (Fig. 1A).

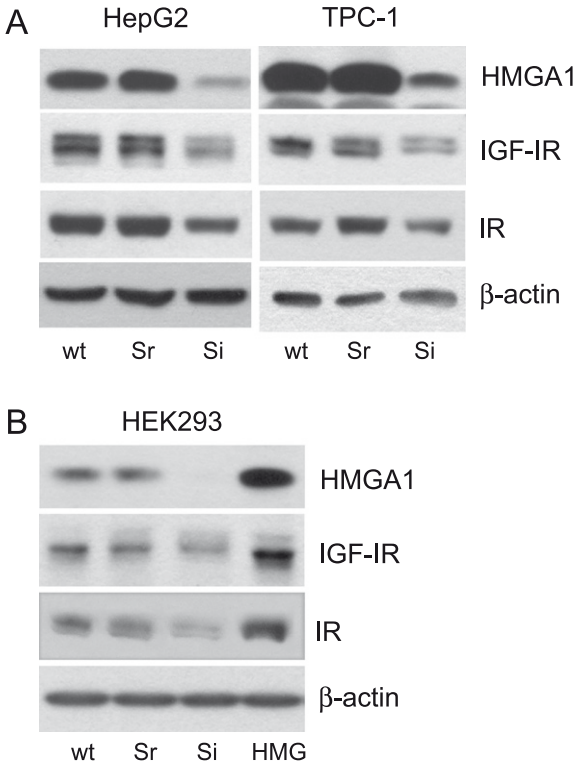

Fig. 1 – HMGA1 silencing and overexpression regulate IGF-IR expression in cells with wild-type p53. (A) HepG2 and TPC-1 cells were transfected with HMGA1 siRNAs (Si); (B) HEK293 cells were transfected with either HMGA1 siRNAs (Si) or a HMGA1 expression plasmid (HMG), as described in Section . Cells left untransfected (wt) or transfected with scramble siRNAs (Sr) were used as controls. Forty-eight hours after transfection, the cells were lysed and analysed by Western blot for the expression of HMGA1, IGF-IR and IR. The same blots were probed with an anti-β-actin antibody to check for protein loading. Blots are representative of three independent experiments.

HMGA1 silencing was associated with a parallel decrease of IGF-IR expression (to 61 ± 4% and 56 ± 7% of control values, respectively; $P < 0.001$) (Fig. 1A) and with a similar reduction of IR expression (to 64.4 ± 5% and 56.6 ± 4% of control values, in HepG2 and in TPC-1, respectively; $P < 0.001$) (Fig. 1A).

We then evaluated whether HMGA1 regulates IGF-IR expression both negatively and positively. To this aim we studied HEK293 cells, which express HMGA1 protein at a lower level than HepG2 or TPC-1, thus allowing us to evaluate not only the effect of HMGA1 silencing but also the effect of HMGA1 overexpression. HEK293 cells were transfected with either HMGA1 siRNA or a HMGA1 expression vector and IGF-IR and IR content was evaluated by immunoblotting. As shown in Fig. 1B, IGF-IR expression was substantially reduced (to 80 ± 6% of control values, $P < 0.004$) by HMGA1 silencing while it was markedly increased (to 216 ± 14% of control values, $P < 0.001$) by HMGA1 overexpression. IR expression followed a similar pattern than IGF-IR: it was reduced to 46 ± 7% ($P < 0.001$) after HMGA1 silencing and increased to 184 ± 12% ($P < 0.002$) after HMGA1 overexpression.

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3.2. *HMGA1 regulates IGF-IR expression also in human cancer cells bearing a null p53 mutation*

In order to evaluate whether HMGA1 regulates IGF-IR expression independently of the presence of p53, we studied two human cancer cell lines null for p53. SW1736 cells, which express a high level of HMGA1, were transfected with HMGA1 siRNA. HMGA1 silencing caused a significant decrease of the IGF-IR protein expression (to 65 ± 8 of control values; $P < 0.05$). A similar, more marked effect was observed on the IR (decreased to 11 ± 4% of control values, $P < 0.001$) (Fig. 2).

In contrast to SW1736 cells, Saos2 cells are characterised by low endogenous levels of HMGA1. These cells were then transfected with increasing doses of a HMGA1 expression plasmid. Forced expression of HMGA1 resulted in a dose-dependent IGF-IR upregulation with a maximal increase to 198 ± 22% of control values ($P < 0.001$) (Fig. 2). IR expression followed a similar pattern with a more marked maximal increase to 281 ± 27% of control values ($P < 0.001$) (Fig. 2).

These results indicate that HMGA1 may modulate IGF-IR expression also independently of p53.

3.3. *Mechanisms of IGF-IR expression regulation by HMGA1 protein*

We then studied the mechanisms of IGF-IR regulation by HMGA1. First, HEK293 cells were transfected with either the proximal IGF-IR promoter, p(–476/+640) Luc or a minimal *igf-ir* promoter fragment, p(–40/+640) Luc, both fused to the luciferase reporter gene in the presence or absence of HMGA1 siRNA (either 25 or 100 nM). HMGA1 silencing reduced the *igf-ir* promoter activity in a dose-dependent manner. The effect of HMGA1 transfection was more marked when using the proximal promoter (approximately 50% decrease after 100 nM siRNA) with respect to cells transfected with the –40/+640 promoter fragment (approximately 20% reduction after 100 nM siRNA) (Fig. 3A).

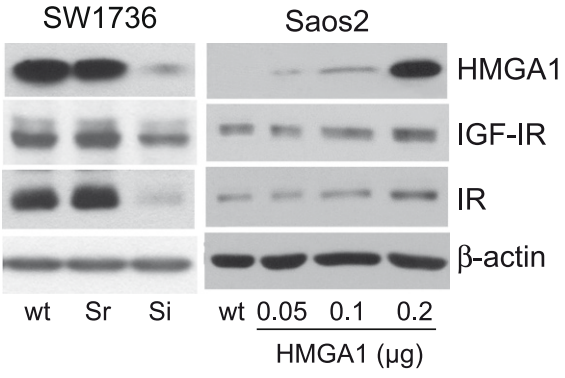

Fig. 2 – HMGA1 silencing and overexpression regulates IGF-IR expression in cells null for p53. SW1736 (left panel) were transfected with HMGA1 siRNAs (Si), whereas Saos2 cells (right panel) were transfected with increasing doses of HMGA1 expression plasmid, as described in Section . Cells left untransfected (wt) or transfected with scramble siRNAs (Sr) were used as controls. Forty-eight hours after transfection, the cells were lysed and analysed by Western blot for HMGA1, IGF-IR and IR expressions. The same blots were probed with an anti-β-actin antibody to check for protein loading. Blots are representative of three independent experiments.

Second, we studied whether HMGA1 protein may modulate the p53 negative regulatory activity on *igf-ir* promoter. To this aim we used p53 null Saos2 cells, which have a low level of endogenous HMGA1. Saos2 cells were transfected with the proximal *igf-ir* promoter reporter and promoter activity was then evaluated in cells cotransfected with either HMGA1 alone or in combination with p53. Transfection with HMGA1 alone increased *igf-ir* promoter activity by approximately 25%, while transfection with p53 alone reduced the promoter activity to less than 50%. When p53 was cotransfected with increasing HMGA1 concentrations, HMGA1 titrated the inhibitory effect of p53 up to the dose of 0.2 μg/dish, when it abolished the effect of p53 (Fig. 3B).

We then evaluated whether increasing HMGA1 protein could also affect the positive regulatory activity of Sp1 on *igf-ir* promoter. Sp1 stimulated *igf-ir* promoter activity by +62 ± 15%. Sp1 activity was blocked by cotransfection with wild-type p53, while it was enhanced by the cotransfection with HMGA1 (Fig. 3C). In the same set of experiments, p53-mediated inhibition of *igf-ir* promoter activity was antagonised by both Sp1 and HMGA1 to a similar extent (Fig. 3C). Taken together, these data suggest that HMGA1 may enhance *igf-ir* promoter activity by both antagonising p53 and potentiating Sp1 activity.

3.4. *HMGA1 interacts with both Sp1 and p53*

We then studied whether HMGA1 protein physically interacts with either Sp1 or p53 or both. To this aim, we cotransfected HEK293 cells with HMGA1, Sp1 and p53 expression vectors. The cells were then lysed, immunoprecipitated with either anti-p53 or anti-Sp1 and immunoblotted with an anti-HMGA1 antibody. As shown in Fig. 4A, HMGA1 protein specifically coimmunoprecipitated with both p53 and Sp1. Data were confirmed by immunoprecipitating cell lysates with either an anti-HMGA1 or an anti-p53 antibody and immunoblotting with an anti-Sp1 antibody (Fig. 4B), and by immunoprecipitating cell lysates with either an anti-Sp1 or an anti-HMGA1 antibody and immunoblotting with an anti-p53 antibody (Fig. 4C).

3.5. *HMGA1 interacts with the igf-ir promoter*

To provide direct evidence that the HMGA1 protein interacts with the *igf-ir* promoter, we used the chromatin immunoprecipitation analysis (ChIP). HEK293T cells were transfected with either a HMGA1-encoding expression vector (HA-HMGA1) or the corresponding empty vector (HA-pcDNA3). The cells were then cross-linked with formaldehyde, lysed, sonicated and immunoprecipitated with a HA antibody, followed by PCR amplification of the IGF-IR promoter DNA (773 bp). As shown in Fig. 5, a specific signal was clearly present in the immunoprecipitated material of HMGA1-transfected, but not of pcDNA3-transfected cells, indicating that HMGA1 binds in vivo to the IGF-IR promoter.

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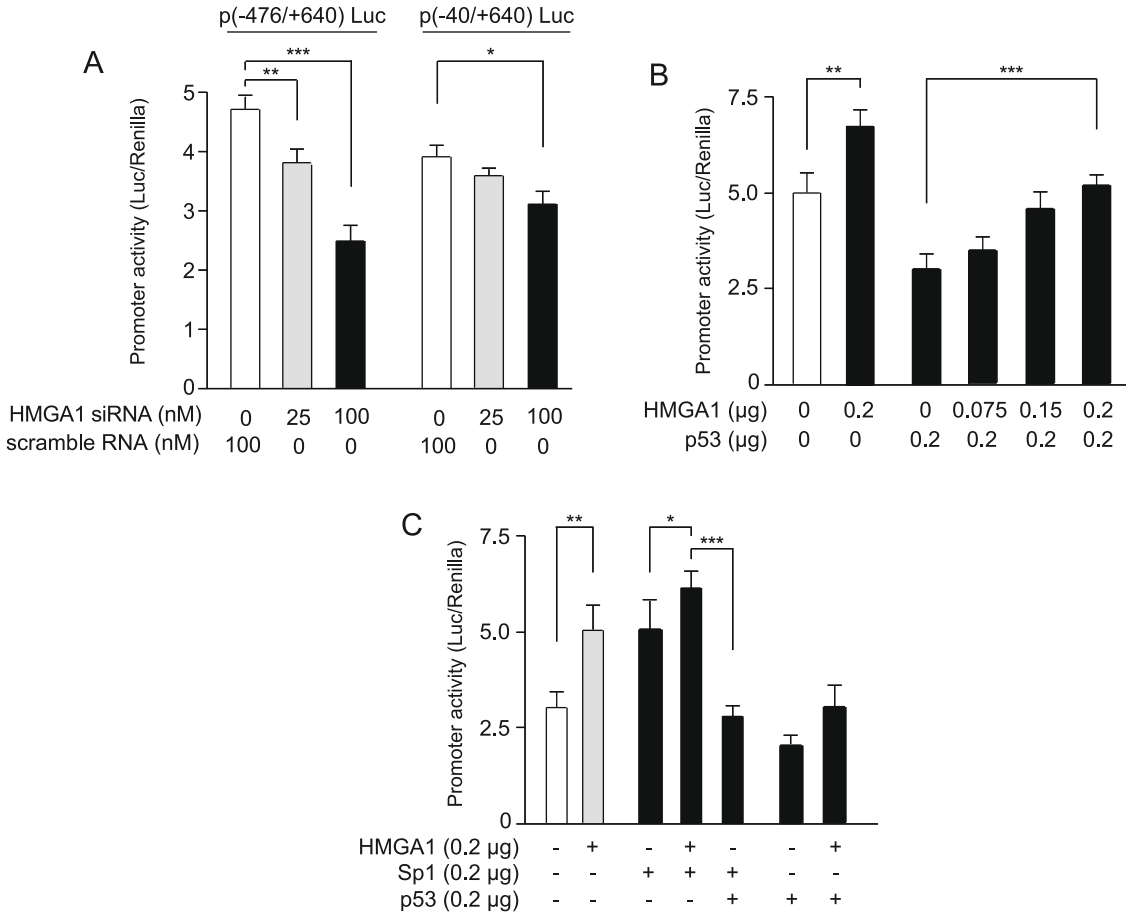

Fig. 3 – IGF-IR promoter activity in Saos2 cells transfected with either HMGA1 or p53 or Sp1 or with different combinations of the transfectants. (A) HEK293 cells were transfected either with the proximal *igf-ir* promoter, p(–476/+640) Luc, or with the minimal *igf-ir* promoter fragment, p(–40/+640) Luc, both fused to the luciferase reporter gene in the presence or absence of HMGA1 siRNA (25 or 100 nM). HMGA1 silencing caused a dose-dependent reduction of *igf-ir* promoter activity, which was more marked when using the proximal promoter than when using the minimal promoter fragment. (B) p53 null Saos2 cells, characterised by low levels of endogenous HMGA1, were transfected with a proximal *igf-ir* promoter construct in the presence or absence of a HMGA1 expression vector and of a wild-type p53 construct, as indicated. Transfection with HMGA1 increased *igf-ir* promoter activity while transfection with p53 decreased it. The inhibitory effect of p53 was progressively impaired by cotransfection with increasing HMGA1 concentrations. (C) Saos2 cells were transfected with constructs encoding for the proximal *igf-ir* promoter, wild-type p53 and Sp1, separately or in combination. Transfection with either HMGA1 or Sp1 alone increased IGF-IR promoter activity, while p53 antagonised it. Cotransfection with HMGA1 increased the stimulating effect of Sp1 while counteracting the inhibitory effect of p53. Data are the mean ± SE of three separate experiments. $^{*}P < 0.01$; $^{**}P < 0.002$; and $^{***}P < 0.0001$.

3.6. *HMGA1 silencing inhibits the growth response to IGF-I and insulin*

In order to evaluate the biological relevance of IGF-IR down-regulation by HMGA1 silencing, we studied TPC-1 cell growth. Cells were exposed to 10 nM IGF-I and the growth was measured by both MTT assay and BrdU incorporation. In control cells, IGF-I increased cell growth by approximately 50% over basal. HMGA1 silencing reduced basal cell growth by approximately 30% and abolished the growth response to IGF-I. Fig. 6 shows data obtained by MTT assay. Very similar data were observed by measuring BrdU incorporation (data not shown). Results similar to IGF-I stimulation were observed with insulin stimulation (Fig. 6).

4. Discussion

Herein we show that HMGA1 protein is a positive regulator of *igf-ir* transcription and that HMGA1 silencing markedly reduces IGF-IR expression in a variety of cancer cells. HMGA1 protein, present at low level in adult differentiated cells, is usually expressed at high level in cancer cells, where it contributes to the maintenance of the malignant/metastatic phenotype.[13,15,16] HMGA1 may represent, therefore, one important factor driving IGF-IR overexpression in cancer cells.

HMGA1 enhances IGF-IR promoter activity and protein expression by multiple mechanisms. One mechanism involves p53, which is a major negative regulator of *igf-ir*

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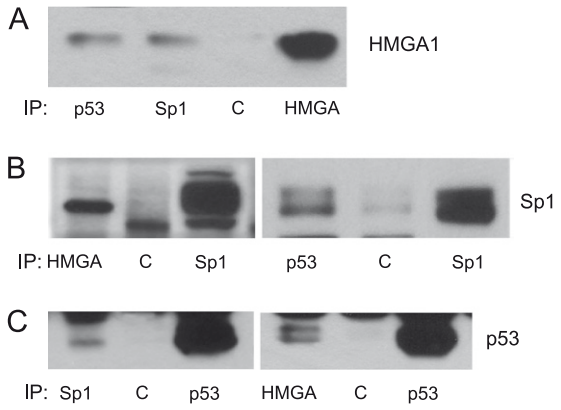

Fig. 4 – HMGA1 protein associates with both p53 and Sp1. HEK293 cells were cotransfected with HMGA1, p53 and Sp1 expression vectors. Forty-four hours after transfection, the cells were lysed and immunoprecipitated with either an anti-p53 or an anti-Sp1 or an anti-HMGA1 antibody and analysed by Western blot. Control samples were immunoprecipitated with an unrelated antibody (C). (A) Filters were blotted with an anti-HMGA1 antibody; HMGA1 (HMGA) protein was found to coimmunoprecipitate with both p53 and Sp1. (B) When the filters were blotted with an anti-Sp1 antibody, Sp1 was found to associate with both HMGA1 and p53. (C) The filters were blotted with an anti-p53 antibody; p53 were found to coimmunoprecipitate with both Sp1 and HMGA1. Blots are representative of three independent experiments.

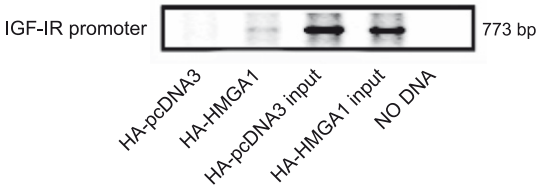

Fig. 5 – ChIP analysis of HMGA1 interaction with the *igf-ir* promoter. ChIP analysis of HMGA1 protein binding to the *igf-ir* promoter DNA. HEK293T cells, transfected with either HA-HMGA1 or control HA-pcDNA3 plasmids, were cross-linked with formaldehyde, lysed, sonicated and immunoprecipitated with a anti-HA antibody, followed by PCR amplification of precipitated chromatin using primers encompassing the *igf-ir* promoter. The position of the 773 bp-amplified fragment is indicated. The input bands represent the amplified PCR products in the absence of antibodies.

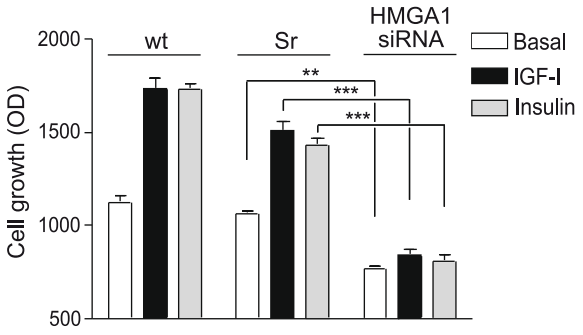

Fig. 6 – HMGA1 silencing inhibits cell growth response to both IGF-I and insulin. TPC-1 cells were transfected with either scramble siRNA or HMGA1 siRNA, as described in methods. Untransfected (wt) or transfected cells were then exposed to either IGF-I or insulin (10 nM) for 48 h and their growth was evaluated by MTT assay. HMGA1 silencing inhibited basal TPC-1 cell growth by approximately 30% and abolished cells growth after stimulation by either IGF-I or insulin. Data are the mean ± SE of three separate experiments. Data are the mean ± SE of three separate experiments. $^{**}P < 0.002$ and $^{***}P < 0.0001$.

transcription. Indeed, inactivating p53 mutations has been implicated as a potential mechanism for IGF-IR dysregulation in cancer cells.[19] We find that HMGA1 protein associates with p53 and impairs the p53 inhibiting effect on the *igf-ir* promoter in a dose-dependent manner. These findings are in close agreement with previous studies showing that HMGA1 protein interacts with the oligomerisation domain of p53 and inhibits p53 apoptotic activity by impairing its ability to oligomerise into functionally active tetramers.[27,28] These studies also showed that HMGA1 silencing leads to increased p53 transcriptional activity.[27] Therefore, HMGA1 overexpression may enhance *igf-ir* gene transcription by causing p53 functional inactivation. Conversely, HMGA1 reduction may cause a parallel decrease of IGF-IR expression through an increase of p53 transcriptional activity. HMGA1 protein, however, is also able to increase *igf-ir* gene transcription and protein expression in p53 null cancer cells, such as Saos2 cells. This finding may be explained by the HMGA1 ability to induce allosteric changes in the regulated promoter regions, thus facilitating the assembly of transcriptional multiprotein complexes and stabilising protein–protein and protein–DNA interactions.[13] Indeed, using the ChIP assay, we found that HMGA1 protein binds to the *igf-ir* promoter. We also found that HMGA1 protein binds Sp1 and enhances its activating effect on the *igf-ir* promoter. Therefore, an additional mechanism for enhanced IGF-IR gene transcription by HMGA1 may involve DNA binding and stabilisation of transcriptional complexes containing Sp1. In keeping with these findings, the enhancing effect of HMGA1 protein was maximal when using the proximal *igf-ir* promoter, containing several Sp1 sites, while it was lower when using the minimal *igf-ir* promoter fragment, lacking Sp1 sites.

The IGF-IR is a close homologue of the IR, which has a predominant role in glucose homeostasis.[29] The IR is also overexpressed in cancer cells, predominantly as isoform A (IR-A), which is a high affinity receptor for both insulin and IGF-II.[21,30] Previous work indicates that *ir* transcription is positively regulated by HMGA1 protein,[18] and although *ir* transcriptional control is different from that of *igf-ir*, both genes share some common regulatory factors, such as p53 and Sp1.[19,20] Now we show that HMGA1 silencing and overexpression have similar effects on IGF-IR and IR protein expression in a variety of transformed cells. We also found that HMGA1 silencing slightly reduce growth in unstimulated cells and

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completely abolish cell growth in response to IGF-I and insulin.

In summary, our data indicate that HMGA1 protein binds to the *igf-ir* promoter and regulates *igf-ir* transcription through multiple mechanisms. The high levels of HMGA1 protein in transformed cells may contribute to IGF-IR and IR dysregulation in cancer and may become a target for therapies aimed at reducing malignant cell response to IGFs and insulin.

Conflict of interest statement

None declared.

Role of funding sources

The funding sources had no role, in the study design; in the collection, analysis and interpretation of data; in the writing of the manuscript and in the decision to submit the manuscript for publication.

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