Nucleosome-binding protein HMGN2 exhibits antitumor activity in human SaO2 and U2-OS osteosarcoma cell lines

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Abstract. High mobility group N (HMGNs) are members of the high mobility group protein family, and are involved in the development and progression of several tumors. HMGN1 and HMGN5 were previously shown to be associated with the bioactivities of osteosarcoma. However, the effects and molecular mechanisms of HMGN2 on osteosarcoma progression remain to be determined. In order to characterize the endogenous expression of HMGN2 in osteosarcoma cell lines, RT-PCR and western blot analysis were performed. Recombinant HMGN2 lentivirus was used to infect the osteosarcoma cell lines with relatively low HMGN2 expression to determine the functional relevance of HMGN2 overexpression in osteosarcoma cell growth and migration *in vitro* and *in vivo*, and to investigate the expression levels of Ki-67, PCNA, cyclin D1 and cyclin E. The results showed that osteosarcoma cell proliferation and migration were significantly reduced by HMGN2, as indicated by cell count and wound-healing assays. Cell apoptosis was markedly induced and HMGN2 increased the sensitivity to chemotherapy. When HMGN2 expression was enhanced, the expression of cyclin D1 and PCNA was downregulated in osteosarcoma cells. In addition, the tumor volumes in SaO2 and U2-OS subcutaneous nude mouse models treated with HMGN2 lentivirus were significantly decreased as compared to those of the GFP group. These results suggested that the enhanced expression of HMGN2 in osteosarcoma cells by HMGN2 lentivirus, exerts inhibitory effects on growth and migration of osteosarcoma cells.

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Introduction

Osteosarcoma accounts for ~20% of all primary bone cancers, and is the second highest cause of cancer-associated mortality in the pediatric age group (1,2). Although modern surgery and neo-adjuvant chemotherapy have developed over the past decades, ~35% of patients are likely to succumb to the disease within 5 years of diagnosis (3). Originating from cells of the osteoblast lineage, osteosarcoma cells exhibit complex and unbalanced karyotypes, characterized by numerous recurrent DNA amplifications as well as chromosomal abnormalities (4).

The high mobility group protein family (HMG) consists of three subfamilies, including the high mobility group B (HMGB), high mobility group A (HMGA) and high mobility group N (HMGN) (5). Each subfamily appears to exert a single characteristic nuclear function, specifically binding to nucleosomes and contributing to the diversity of chromatin function (6). Among them, HMGN2 is an abundant, highly conserved cell protein, widely known as a nuclear DNA-binding protein, which stabilizes nucleosome formation and facilitates gene transcription.

Findings of previous studies demonstrated that HMGN2 was one of the most abundant non-histone nuclear proteins in vertebrates and invertebrates (6,7). Subsequently, it was found that HMGN2 was mainly involved in the growth of tumor vascular endothelia and played an important role in tumorigenesis (8). The aberrant expression of HMGN2 was correlated with several tumors (9,10). *In vitro*, HMGN2 was released by the stimulation of IL-2 in human peripheral blood mononuclear leukocytes (8). HMGN2 protein was demonstrated to inhibit growth and induce apoptosis in the Tca8113 oral squamous cell carcinoma cell line (11). *In vivo*, HMGN2 significantly inhibited growth of the transplanted tumor in nude mice (12).

Therefore, HMGN2 is a newly identified gene associated with cancer growth and metastasis, representing a new therapeutic target for the treatment of cancer. However, the effects and molecular mechanisms of HMGN2 on osteosarcoma progression have not yet been comprehensively explored. In the present study, we examined the endogenous expression of HMGN2 in osteosarcoma cell lines using RT-PCR and

western blot analysis. HMGN2 lentivirus was used to infect the osteosarcoma SaO2 and U2-OS cell lines with a relatively low HMGN2 expression to determine the functional relevance of HMGN2 overexpression in osteosarcoma cell growth and migration *in vitro*, and to examine the underlying signaling pathway involved in the progression of osteosarcoma.

Materials and methods

Materials. MG63, HOS, SaO2 and U2-OS osteosarcoma cell lines were purchased from the American Type Culture Collection (ATCC, Vanassas, MA, USA). HMGN2 lentivirus vector, negative control vector GFP and virion-packaging elements were purchased from Genechem (Shanghai, China). All the antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA).

Pharmaceuticals and reagents. TRIzol reagent, Lipofectamine 2000, Double stain apoptosis detection kit (Annexin V-FITC/PI), Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco Invitrogen Corporation (Grand Island, NY, USA). 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfo-phenyl)-2H-tetrazolium (WST-8) was purchased from Beyotime (Beyotime, Haimen, China) and SYBR-Green master mixture was purchased from Takara (Otsu, Japan).

Overexpression of HMGN2. Human-HMGN2 cDNA was prepared by RT-PCR (Fig. 1A) and inserted into the *Bam*HI and *Xho*I restriction sites of the pGC-FU-3FLAG vector plasmid (Addgene, Cambridge, MA, USA). For the stable overexpression of HMGN2, HEK293T cells were plated in 75-cm^2 culture flasks and transfected with 10 μg HMGN2 or GFP (control) lentivirus vectors. The medium was changed and the viral supernatant was harvested 48 h later. Viral-containing medium was collected and passed through 0.45-μm syringe filters. SaO2 and U2-OS cells were incubated with the lentivirus supernatant for 24 h and selected with 2 μg/ml puromycin (Sigma, St. Louis, MO, USA) according to the manufacturer's instructions. The clone in which the HMGN2 lentivirus vectors transfected was designated as the HMGN2 group, the negative control GFP transfected vectors was designated as the GFP group and SaO2 and U2-OS osteosarcoma cells were designated as the CON group.

RT-qPCR. To quantitatively determine the expression level of HMGN2 in osteosarcoma cell lines, HMGN2 mRNA expression was determined by RT-qPCR using SYBR-Premix Ex Taq (Takara, Japan) and an ABI Prism 7500 sequence detection system (Applied Biosystems, USA). Total RNA of each clone was extracted with TRIzol according to the manufacturer's instructions. The genes were amplified using specific oligonucleotide primers and human β-actin gene was used as an endogenous control. The PCR primer sequences used were: HMGN2, forward: 5'-CCAGCCATCAGCCATGAGGGT-3' and reverse: 5'-GGAGCCCTTTCTGAATCCGCA-3'); β-actin, forward: 5'-GCGGGAAATCGTGCGTGACATT-3' and reverse: 5'-GGCAGATGGTCGTTTGGCTGAATA-3'. Data were analyzed using the comparative Ct method ($2^{-\Delta\Delta Ct}$). Three separate experiments were performed for each clone.

Western blot assay. Treated cells were collected and extracted using an assay kit (Beyotime, China) and the concentration was determined by using a Bio-Rad protein assay (Hercules, CA, USA). Equal amounts of cell extracts were separated on SDS-PAGE gels according to the molecular weight of the tested proteins. Separated protein bands were transferred onto polyvinylidene fluoride membranes and blocked in 5% skimmed milk powder. The primary antibodies against HMGN2, cyclin E, cyclin D1, PCNA, and Ki-67 (all from Santa Cruz Biotechnology) were diluted according to the instructions and incubated overnight at 4˚C. Horseradish peroxidase-linked secondary antibodies were added at a dilution ratio of 1:1,000, and incubated at room temperature for 2 h. The membranes were washed with phosphate-buffered saline three times and the immunoreactive bands were visualized using an ECL-Plus kit according to the manufacturer's instructions [Multi Sciences (Lianke) Biotech Co., Ltd., Hangzhou, China]. The relative protein level in different cell lines was normalized to β-actin concentration. Three separate experiments were performed for each clone.

Cell proliferation assay. Cell proliferation was analyzed with the cell count assay using WST-8 kits. Briefly, cells infected with HMGN2 were incubated in 96-well plates at a density of $1x10^5$ cells/well with DMEM with 10% FBS. The cells were treated with 10 μl WST at 6, 12 and 24 h. The color reaction was measured at 570 nm with enzyme immunoassay analyzer (Bio-Rad Laboratories, Hercules, CA, USA). The proliferative activities were calculated for each clone.

Cell cycle analysis. For the cell cycle analysis, harvested cells were centrifuged at 1,000 x g for 5 min, washed with phosphate buffered saline and fixed in 70% ethanol overnight. The cells were then treated with 100 μg/ml propidium iodide plus RNase (10 μg/ml) for 30 min. The cell-cycle phase distribution was determined by analytical DNA flow cytometry (FACSCalibur; BD Biosciences, Becton-Dickinson, San Jose, CA, USA) as described by Evans *et al* (13).

Wound-healing assay. SaO2 and U2-OS cells were plated in each well of a 6-well culture plate and allowed to grow to 90% confluence. Treatment with HMGN2 lentivirus was then performed. The following day, a wound was created using a 10 μl micropipette tip. The migration of cells towards the wound was monitored daily, and images were captured at time intervals of 24 h.

In vivo tumor xenograft studies. Three mice were injected subcutaneously with $1x10^8$ SaO2 and U2-OS cells in 50 ml of PBS pre-mixed with an equal volume of Matrigel matrix (Becton Dickinson). When the tumor size reached ~5 mm in length, sections were surgically removed, cut to 1-mm^3, and re-seeded individually into other mice. When the tumor size reached ~5 mm in length, the mice were randomly assigned to the GFP and HMGN2 groups, in which 15 ml of lentivirus was injected into subcutaneous tumors using a multi-site injection format. The injections were repeated on the third day after initial treatment. The tumor volume was measured every three days with a caliper, using the formula volume = $(\text{length} \times \text{width})^2/2$.

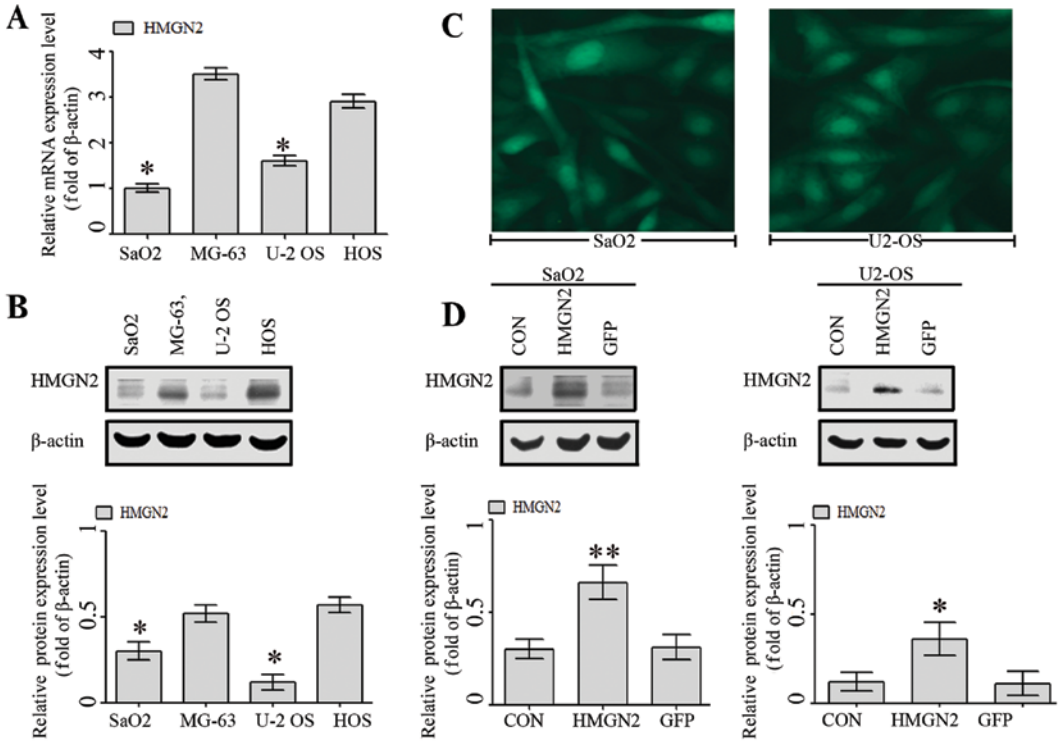

Figure 1. The endogenous expression of HMGN2 in osteosarcoma cell lines and SaO2 and U2-OS cell lines overexpressed HMGN2 construction. Different levels of (A) mRNA and (B) protein expression of HMGN2 were detected in SaO2, MG-63, U2-OS, and HOS cell lines, but the expression levels of HMGN2 were significantly lower in SaO2 and U2-OS cell lines than those in MG-63 and HOS cell lines. (C) A recombinant lentivirus vector encoding the *HMGN2* gene was constructed for infecting SaO2 and U2-OS cell lines. The infection efficiency of HMGN2 lentivirus (MOI =50) was >80% under fluorescence microscopy. (D) The protein levels of HMGN2 were determined by western blotting when SaO2 and U2-OS cells transfected with HMGN2 lentivirus vectors for 24 h. A significant increase of HMGN2 expression was observed in the HMGN2 group ($^{*}P<0.05$ and $^{**}P<0.01$).

Statistical analysis. The results obtained were expressed as the mean ± standard error values from at least three independent experiments. One-way analysis of variance (ANOVA) was used to analyze the differences between groups. The LSD method of multiple comparisons was used when the probability for ANOVA was statistically significant. P<0.05 was considered to indicate statistical significance.

Results

Endogenous expression of HMGN2 in osteosarcoma cell lines. The endogenous expression of HMGN2 in human osteosarcoma SaO2, MG-63, U2-OS and HOS cell lines was evaluated using RT-qPCR and western blot analysis. As shown in Fig. 1A and B, there were different levels of mRNA and protein expression of HMGN2 in SaO2, MG-63, U-2 OS, and HOS cell lines, while the expression levels of HMGN2 were significantly reduced in the SaO2 and U-2 OS cell lines than those in the MG-63 and HOS cell lines.

Since HMGN2 exhibited a low expression in SaO2 and U-2 OS cell lines, the cell lines were selected as the infective objects of HMGN2 lentivirus. The infection efficiencies of HMGN2 (at a multiplicity of infection =50) in SaO2 and U-2 OS cell lines were >80% under fluorescence microscopy (Fig. 2C). To confirm that HMGN2 successfully transfected into SaO2 and U-2 OS cells, western blot analysis was applied. After 48 h following HMGN2 or GFP lentivirus infection and the expression of HMGN2 protein was significantly increased in the HMGN2 group (Fig. 1D).

HMGN2 inhibits osteosarcoma cell growth. Deregulated cell proliferation is a hallmark of cancer (16). To determine the effect of HMGN2 overexpression on SaO2 and U-2 OS cell growth, we investigated the proliferative activities by WST assay. Overexpression of HMGN2 significantly reduced the proliferative activities of SaO2 and U-2 OS cells compared with the GFP and CON groups (Fig. 2A). In addition, PCNA and Ki-67, which were indicators for cell proliferation, were examined by western blot assay to determine whether HMGN2 overexpression suppressed cell growth through translational repression. The expression of PCNA protein was significantly decreased in Lenti-HMGN2 group compared with the CON and GFP groups (P<0.01) while Ki-67 did not alter among these groups (Fig. 2B). These data suggested that the overexpression of HMGN2 may inhibit osteosarcoma cell proliferation through the downregulation of PCNA expression.

Effect of HMGN2 on osteosarcoma cycle distribution. The cycle distribution of SaO2 and U-2 OS cells was also analyzed. As shown in Fig. 3A, the percentage of untransfected SaO2 and U-2 OS cells in S phase was 52.1 and 58%, respectively, whereas the percentage of SaO2 and U-2 OS cells transfected with HMGN2 in S phase was 35.5 and 33%, respectively. In addition, 10 and 15% of untreated SaO2 and U-2 OS cells were in the G2/M phase compared with 5 and 8% of cells transfected with HMGN2 lentivirus. These results indicated that cell cycle was arrested in G0/G1 phase in HMGN2 group compared with the CON and GFP groups. Subsequently, cyclin D1 and cyclin E, two regulators of cell cycle progression

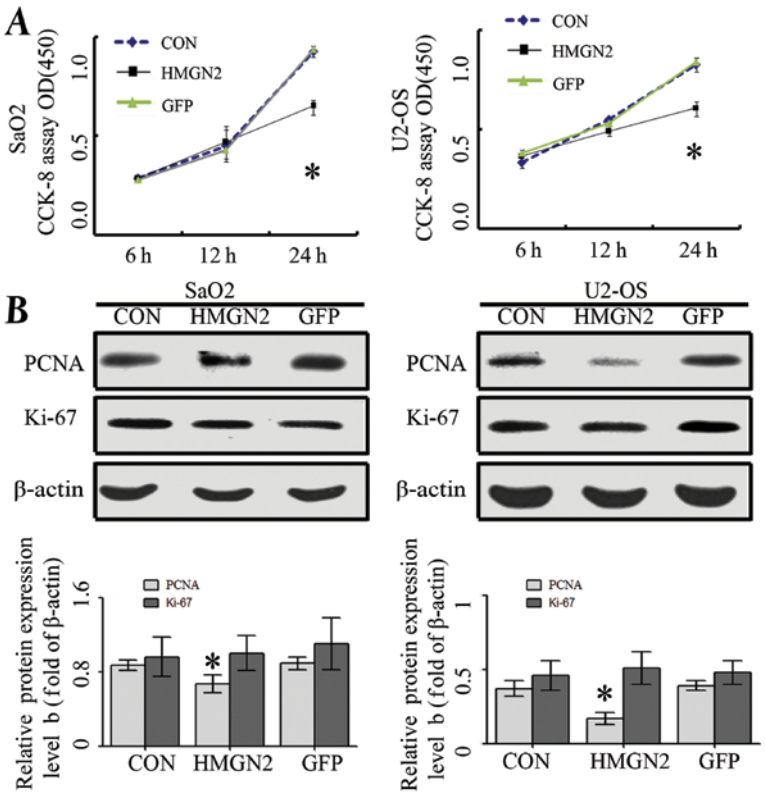

Figure 2. Effect of HMGN2 on proliferation and its effect on PCNA and Ki-67 expression. (A) WST-8 assay indicated that a significantly reduction of the proliferative activity in the HMGN2 group compared with those in the GFP and CON groups. No difference was found between the GFP and CON groups. (B) The expression of PCNA and Ki-67 was examined by western blot assay. The results indicated that the amount of PCNA expression was significantly decreased in the HMGN2 group compared with the GFP and CON groups in SaO2 and U-2 OS (*P<0.05). By contrast, the expression of Ki-67 was similar among these groups.

from G1 to S phase, were assessed by western blotting (17). The results showed that only HMGN2 led to a decreased level of cyclin D1, whereas no significant changes were identified in the levels of cyclin E (Fig. 3B), suggesting that HMGN2 modulates the cell cycle through the regulation of cyclin D1.

Effect of HMGN2 on osteosarcoma cell invasion and metastasis. To determine the effect of HMGN2 on osteosarcoma cell invasion and metastasis, Transwell assay and wound-healing assay were carried out. As was shown in Fig. 4A, the migrative ability of SaO2 and U-2 OS cells in the HMGN2 group was lower than that in the CON and GFP groups. However, there were no significant differences between the CON and GFP groups. Furthermore, a Transwell assay was performed to determine the ability of cells to invade a matrix barrier. The representative micrographs of Transwell filters are shown in Fig. 4B. The invasive cell count demonstrated that invasive potential was significantly reduced in the HMGN2 group relative to the CON and GFP groups.

HMGN2 increases apoptosis and sensitivity to chemotherapy. The action mechanism of many anticancer factors is based on their ability to induce apoptosis. Consequently, SaO2 and U-2 OS osteosarcoma cells treated with HMGN2 lentivirus underwent apoptosis as their mode of cell death. At the end of the incubation period for 24, 48 and 72 h, the osteosarcoma cells were stained using an Annexin V-FITC/PI detection kit. As shown in Fig. 4C, the number of SaO2 and U-2 OS apoptotic cells in the HMGN2 group significantly increased compared with that in the GFP and CON groups at various time points. To further investigate whether HMGN2 affected the sensitivity to chemotherapy, the apoptosis in HMGN2-overexpressed osteosarcoma cells was induced by chemotherapy. A significant increase of apoptosis incidence was detected in cells treated with Dox, Cis, and Mtx for 24 h (Fig. 4D). Overexpression of HMGN2 resulted in a further increase of apoptosis induced by these anticancer agents, suggesting that HMGN2 increased the sensitivity to chemotherapy.

Antitumor effect of lenti-HMGN2 in the osteosarcoma xenograft model. The *in vitro* experiments confirmed that HMGN2 efficiently inhibited the growth and migration of U2-OS and SaO2 cells. However, whether HMGN2 has the same inhibitory effect on *in vivo* osteosarcoma remains to be determined. We investigated the antitumor effect of HMGN2 *in vivo* using SaO2 and U2-OS xenograft models. The mean volumes of SaO2 and U2-OS xenograft tumors were 52.3±10.5 and 35.5±8.6 mm^3 in the experimental mice prior to treatment. On day 14, the average volumes of SaO2 and U2-OS xenograft tumors were significantly smaller in the HMGN2 group than those in the CON and GFP groups (Fig. 5A). During the whole tumor growth period, the tumor growth activity was measured. Tumors treated with HMGN2 lentivirus grew substantially slower than the GFP group (Fig. 5B). When the tumors were harvested, the average weights of SaO2 and U-2 OS xenograft tumors in HMGN2 group were significantly lighter than those

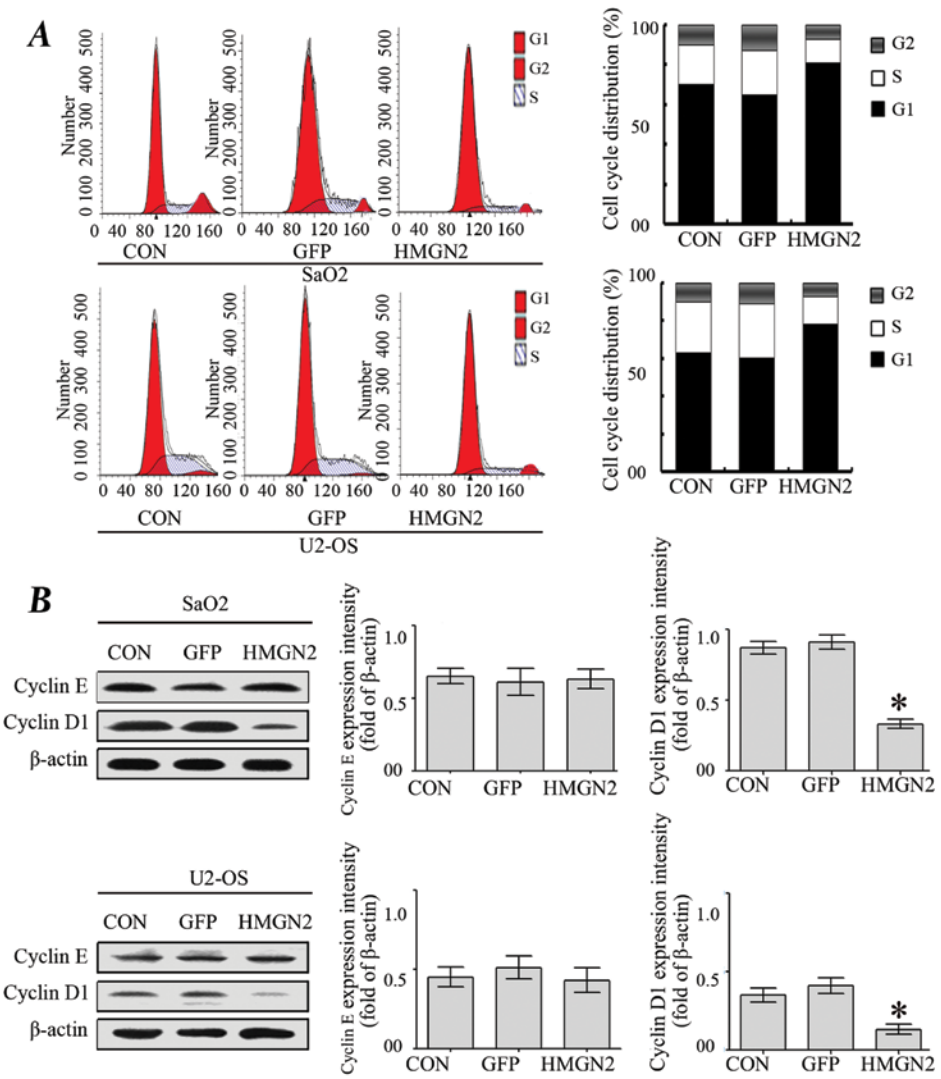

Figure 3. Effect of HMGN2 on cell cycle distribution in SaO2 and U-2 OS cell lines. (A) Cell cycle distribution was analyzed by flow cytometric analysis. The percentage of SaO2 and U-2 OS cells in HMGN2 group decreased in S phases population while the percentage of cells in G0/G1 phase was similar among these groups. However, no significant differences were observed between GFP and CON groups. (B) Western blot analysis was performed at 48 h recovery to measure the expression of cyclin E and cyclin D1, showing that an obvious decrease of cyclin D1 expression was observed in the HMGN2 group compared with the GFP and CON groups (*P<0.05), while cyclin E was not altered by HMGN2 in SaO2 and U2-OS cell lines (*P<0.05). However, no difference was found between the GFP and CON groups in SaO2 and U2-OS cell lines (P>0.05).

in the GFP group (**P<0.01; Fig.5C). These results *in vivo* indicated that overexpression of HMGN2 was able to inhibit SaO2 and U-2 OS cell growth.

Discussion

HMGNs were initially regarded as transcription coregulators, however, their roles in DNA repair and cancer progression were determined using HMGN1 knockout mice (14). In addition to HMGN1, the expression of HMGN5 (formerly NSBP1) was found to be one of the significant factors in the prognosis of bone tumor (15), breast cancer (16) and prostate cancer (17). Collectively, results of those studies suggested that HMGNs were involved in osetosarcoma cell progression and exhibited characteristics of a tumor-suppressor gene. However, whether HMGN2 was expressed in osteosarcoma and the role of HMGN2 was not previously reported. The present study investigated the activity of HMGN2 in the MG-63, HOS, SaO2 and U2-OS osteosarcoma cell lines. To the best of our knowledge, the present study documented for the first time the endogenous expression of HMGN2 in osteosarcoma cell lines, and demonstrated that the expression levels of HMGN2 were significantly lower in SaO2 and U2-OS cell lines than those in MG-63 and HOS cell lines, which were selected as the infective objects of HMGN2 lentivirus. In pilot studies, the infectious efficiency of HMGN2 in SaO2 and U2-OS cell lines was extremely high, and a marked increase of HMGN2 expression was observed in the HMGN2 group compared with the GFP and CON groups. HMGN2 is mainly expressed in vertebrates and invertebrates (6,7) and functions as a modifier of the nucleosomal organization. In addition to the above function, the findings in our study demonstrated that overexpression of HMGN2 significantly reduced the proliferative activities of SaO2 and U2-OS cell lines in a time dependent manner. As shown in our findings, the possible underlying mechanism is that HMGN2, via downregulation of PCNA and cyclin D1 expression, inhibits osteosarcoma cell proliferation.

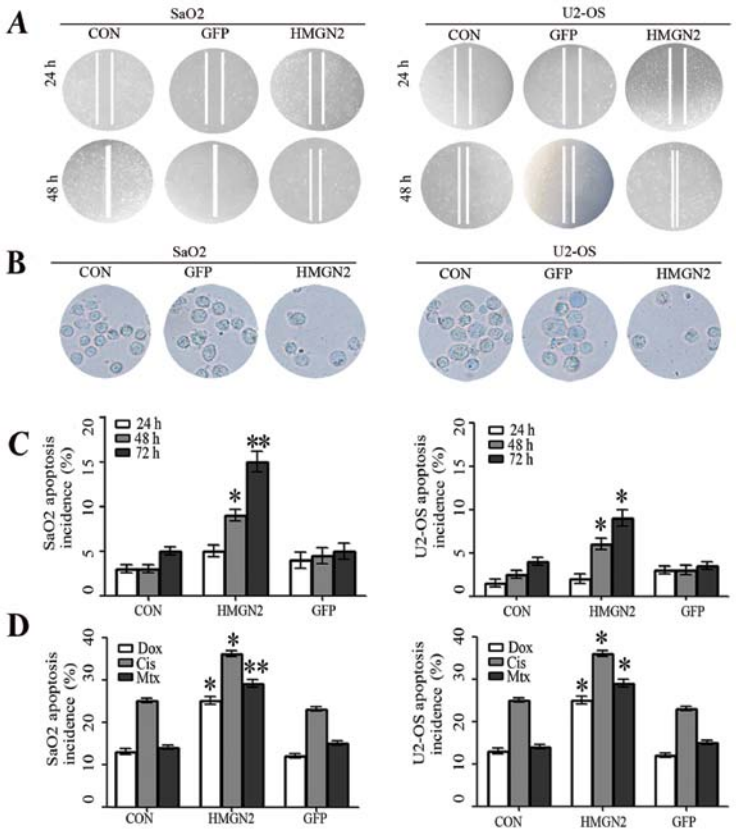

Figure 4. Inhibition of osteosarcoma cell migration and invasiveness by HMGN2. (A) Wound-healing assay showed that the migration capacity of SaO2 and U2-OS cells in the HMGN2 group were markedly lower than those in the GFP and CON groups. (B) Transwell invasion assay for the transmembrane ability of each group of cells. The ability in HMGN2 group was markedly decreased as compared with the GFP and CON groups. (C) The apoptosis of SaO2 and U2-OS cells was analyzed by flow cytometry (Annexin V-FITC/PI) at the end of the incubation period for 24, 48 and 72 h. The apoptosis incidence increased in a time-dependent manner and HMGN2 resulted in a further increase (*P<0.05, **P<0.01), although no difference was found between the GFP and CON groups (P>0.05). (D) Annexin V-FITC/PI staining for apoptosis induced by chemotherapy agents. SaO2/CON, SaO2/GFP, SaO2/HMGN2, U2-OS/CON, U2-OS/GFP, and U2-OS/HMGN2 cells were treated with Dox (0.2 mg/ml), Cis (20 mmol/l), and Mtx (50 mmol/l) for 24 h and the apoptosis incidence was quantified by flow cytometer. Overexpression of HMGN2 resulted in a further increase of apoptosis induced by Dox, Cis and Mtx. (*P<0.05, **P<0.01 vs. CON group).

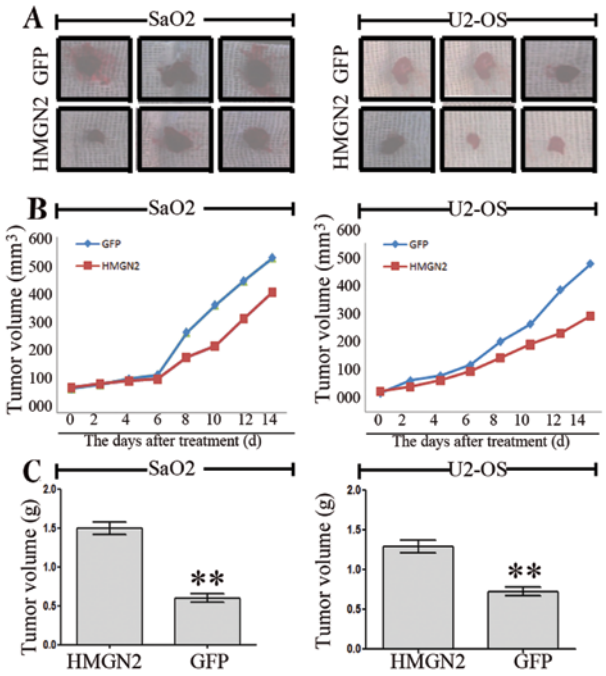

Figure 5. Antitumor effect of HMGN2 in the osteosarcoma SaO2 and U2-OS xenograft models. (A) On day 14, the average volumes of SaO2 and U2-OS xenograft tumors were measured and found to be significantly smaller in HMGN2 group than those in the GFP group. (B) During the whole tumor growth period, the tumor growth activity was measured, showing that the tumors treated with HMGN2 lentivirus grew substantially slower than the GFP group. (C) On day 14, the average weights of SaO2 and U2-OS xenograft tumors were measured and found to be significantly lighter in HMGN2 group than those in the GFP group (**P<0.01).

More importantly, HMGN2 has been confirmed to prevent migration and invasiveness and induce apoptosis, which suggesting that HMGN2 is a tumor suppressor and apoptosis regulator in osteosarcoma. However, the response to HMGN2 is not concordant among all types of cancer. The biological response of cancer cells to HMGN2 may depend, not only on the particular cell type, but also on the presence of other factors that remain to be defined. Our gain-of-function studies *in vitro* and *in vivo* using HMGN2 lentivirus revealed a significant decrease in growth and migration and an increase in apoptosis in SaO2 and U2-OS cells, suggesting that HMGN2 may function as a tumor suppressor in osteosarcoma.

To the best of our knowledge, this is the first study to provide data demonstrating that HMGN2 has an inhibitory effect on growth and migration of osteosarcoma cells. However, limited evidence was obtained regarding the use of osteosarcoma cells in the two cell lines. Investigations using more cell lines and primary tumor are therefore crucial to confirm the findings of this study. In conclusion, the present results have shown that the enhanced expression of HMGN2 in osteosarcoma cells by HMGN2 lentivirus exerts inhibitory effects on growth and migration of osteosarcoma cells. HMGN2 as a tumor suppressor may provide a novel approach to human osteosarcoma treatment.

References

1. Huang J, Ni J, Liu K, *et al*: HMGB1 promotes drug resistance in osteosarcoma. Cancer Res 72: 230-238, 2012.
2. Clark JC, Dass CR and Choong PF: A review of clinical and molecular prognostic factors in osteosarcoma. J Cancer Res Clin Oncol 134: 281-297, 2008.
3. Meyers PA, Schwartz CL, Krailo MD, *et al*: Osteosarcoma: the addition of muramyl tripeptide to chemotherapy improves overall survival - a report from the Children's Oncology Group. J Clin Oncol 26: 633-638, 2008.
4. Lau CC, Harris CP, Lu XY, *et al*: Frequent amplification and rearrangement of chromosomal bands 6p12-p21 and 17p11.2 in osteosarcoma. Genes Chromosomes Cancer 39: 11-21, 2004.
5. Musselman CA and Kutateladze TG: Methyl fingerprinting of the nucleosome reveals the molecular mechanism of high-mobility group nucleosomal-2 (HMGN2) association. Proc Natl Acad Sci USA 108: 12189-12190, 2011.
6. Bustin M: Regulation of DNA-dependent activities by the functional motifs of the high-mobility-group chromosomal proteins. Mol Cell Biol 19: 5237-5246, 1999.
7. Postnikov YV, Herrera JE, Hock R, Scheer U and Bustin M: Clusters of nucleosomes containing chromosomal protein HMG-17 in chromatin. J Mol Biol 274: 454-465, 1997.
8. Porkka K, Laakkonen P, Hoffman JA, Bernasconi M and Ruoslahti E: A fragment of the HMGN2 protein homes to the nuclei of tumor cells and tumor endothelial cells in vivo. Proc Natl Acad Sci USA 99: 7444-7449, 2002.
9. Srikantha T, Landsman D and Bustin M: Retropseudogenes for human chromosomal protein HMG-17. J Mol Biol 197: 405-413, 1987.
10. Spieker N, Beitsma M, van Sluis P, *et al*: An integrated 5-Mb physical, genetic, and radiation hybrid map of a 1p36.1 region implicated in neuroblastoma pathogenesis. Genes Chromosomes Cancer 27: 143-152, 2000.
11. Hu A, Dong X, Liu X, *et al*: Nucleosome-binding protein HMGN2 exhibits antitumor activity in oral squamous cell carcinoma. Oncol Lett 7: 115-120, 2014.
12. Liu X, Dong X, Zhang Y, *et al*: Inhibitory effects of high mobility group chromosomal protein N2 on human tongue carcinoma transplanted in nude mice. Hua Xi Kou Qiang Yi Xue Za Zhi 32: 5-8, 2014 (In Chinese).
13. Evans DL, Bishop GR and Jaso-Friedmann L: Methods for cell cycle analysis and detection of apoptosis of teleost cells. Methods Cell Sci 22: 225-231, 2000.
14. Birger Y, Catez F, Furusawa T, *et al*: Increased tumorigenicity and sensitivity to ionizing radiation upon loss of chromosomal protein HMGN1. Cancer Res 65: 6711-6718, 2005.
15. Zhou X, Yuan B, Yuan W, Wang C, Gao R and Wang J: The expression and clinical significance of high mobility group nucleosome binding domain 5 in human osteosarcoma. Tumour Biol 35: 6539-6547, 2014.
16. Li DQ, Hou YF, Wu J, *et al*: Gene expression profile analysis of an isogenic tumour metastasis model reveals a functional role for oncogene AF1Q in breast cancer metastasis. Eur J Cancer 42: 3274-3286, 2006.
17. Huang C, Zhou LQ and Song G: Effect of nucleosomal binding protein 1 in androgen-independent prostatic carcinoma. Zhonghua Yi Xue Za Zhi 88: 657-660, 2008 (In Chinese).