The expression and clinical significance of high mobility group nucleosome binding domain 5 in human osteosarcoma

Xuhui Zhou · Bo Yuan · Wen Yuan · Ce Wang · Rui Gao · Junyan Wang

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Abstract The high mobility group nucleosome binding domain 5 (HMGN5) is a member of the high mobility group proteins family and highly expressed in multiple malignancies. Nevertheless, the role of HMGN5 in osteosarcoma remains unclear. This study aimed to investigate the expression and clinical significance of HMGN5 in human osteosarcoma, confirm the oncogenic role of HMGN5 and explore the mechanism by which HMGN5 contributes to invasion and metastasis. HMGN5 expression was detected in osteosarcoma tissues and corresponding adjacent non-cancerous tissues (ANCTs) from 52 patients by immunohistochemical (IHC) assay and the clinicopathologic characteristics of all patients were also recorded. Next, osteosarcoma cells were transfected by HMGN5 RNA interference and cell viability, apoptosis and invasion were detected by cell vitality test, flow cytometry and transwell assay, respectively. As a result, IHC assay showed strong immunized activity of HMGN5 in the nucleus in all osteosarcoma tissues compared with the ANCT (53.5±4.3 % vs. 17.0±3.9 %, \( P < 0.01 \)). HMGN5 expression level was associated with pathologic staging and TNM staging. Knockdown of HMGN5 induced cell cycle arrest, inhibited invasion and increased sensitivity to doxorubicin–induced cell apoptosis in U2-OS and SaO2 cells. Western blot analysis demonstrated that there were increased expressions of cleaved caspase-3, cleaved PARP and decreased expressions of PCNA, PI3Kp85α, p-AKT, MMP-9, and cyclin B1 in U2-OS and SaO2 cells depleted of HMGN5. HMGN5 plays oncogenic role in osteosarcoma by promoting cell proliferation and invasion, and could be exploited as a target for therapy in osteosarcoma.

Keywords Nucleosome binding protein · Osteosarcoma · Proliferation · Invasion · AKT

Introduction

Osteosarcoma is one of the most common cancer worldwide, comprising 2.4 % of all malignancies in pediatric patients and approximately 20 % of all primary bone cancers [1]. Although therapeutic interventions for patients with osteosarcoma have improved over the past few decades, the 5-year overall survival is still low (about 60 %) [2]. One of the primary reasons for the poor prognosis for this disease is that patients barely respond to chemotherapy [3]. Thus, a better understanding the molecular mechanism of host tumor interaction is essential for the development of novel treatment strategies for this disease.

High mobility group nucleosome binding domain 5 (HMGN5) is a member of the high mobility group proteins family and widely expressed in higher eukaryotes. HMGN5 contains a functional nucleosomal binding domain (NBD) and a negatively charged C terminus [4]. HMGN5, mediates by NBD, bind to the nucleosomes and involves in transcriptional activation [5]. HMGN5 protein was first identified as a factor that induced differentiation in mouse embryonic stem cell [6]. HMGN5 also played an important role in tumorigenesis and its aberrant expression was correlated with several tumors. For example, an increase of HMGN5 protein was observed in prostate cancer [7] and squamous cell carcinoma in human [8], adenocarcinoma in mice [9] and in the highly metastatic MDA-MB-435HM breast cancer cell line [10], suggesting a role for HMGN5 in tumorigenesis. In addition, it was reported that the expression of HMGN5 was positively correlated with
tumor grade, pathologic stage, and lymph node metastasis in human bladder cancer. Furthermore, knockdown of HMGN5 could inhibit the proliferation and invasion of bladder cancer cells [11]. Consistently, targeting HMGN5 by RNA interference also inhibited the growth and metastasis of renal cell carcinoma cells in vitro and in vivo [12]. Therefore, HMGN5 is a newly identified gene associated with cancer growth and metastasis, representing a new therapeutic target for the treatment of cancer [13].

However, the clinical significance and molecular mechanism of HMGN5 expression in osteosarcoma have not yet been comprehensively explored. In the present study, the expression and clinical significance of HMGN5 protein in osteosarcoma were assessed using immunohistochemical (IHC) assay through tissue microarray procedure. Next, we assessed the effect of RNA interference-mediated knockdown of HMGN5 on the proliferation, cell cycle progression, invasion and chemosensitivity of osteosarcoma cells and further explored the signaling pathways necessary for HMGN5’s effect on progression of osteosarcoma cells.

Materials and methods

Clinical samples and data

Tissue microarray was prepared for IHC test from a total of 52 consecutive cases of human osteosarcoma tissues and corresponding adjacent non-cancerous tissue (ANCTs) operated in Department of Orthopedic Surgery, Changzheng Hospital, Second Military Medical University from January 2003 to December 2009. The pathologic staging was made according to American Joint Committee on Cancer (AJCC) TNM staging system. The use of the tissue samples and clinical data was approved by the Medical Ethics Committee of Second Military Medical University, and the patients’ clinical and pathologic data are summarized in Table 1.

Cell culture and Regents

The human osteosarcoma U2-OS and SaO2 cell lines used in the experiments were from Institute of Biochemistry and Cell Biology (Shanghai, China). U2-OS and SaO2 cells were cultured in DMEM medium supplemented with 10 % heat-inactivated FBS (Gibco, Uruguay), 100U/ml of penicillin and 100 μg/ml of streptomycin. They were cultured in a humidified atmosphere containing 5 % CO₂ at 37°C. Lipofectamine 2000 was purchased from Invitrogen (Carlsbad, CA, USA). Cell Count Kit-8 (CCK-8) and ECL-PLUS/Kit was purchased from Beyotime (Haimen, China); M-MLV Reverse Transcriptase was purchased from Promega (Madison, USA). All antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

RNA-mediated knockdown of HMGN5 and transfection

U2-OS and SaO2 cell lines were transfected with small interfering RNAs (siRNA) targeting specifically at the human HMGN5 genes. siRNA duplexes were designed and synthesized by Genechem (Shanghai, China). A recombinant plasmid contained the target sequence of 5'-ATGAGAAAGGAGAAGATGC-3' (siHMGN5) and a negative control plasmid contained non-silencing control sequence (lack of complementary sequences in the human genome, the negative control plasmids) of 5’-GAAGAATATCGAAGGAAGA -3’.

HMGN5 siRNA or the negative control plasmids (siCON) were transfected into U2-OS and SaO2 cell lines using Lipofectamine 2000 (Carlsbad, USA). Positive stable transfectants were selected and expanded for further study. U2-OS and SaO2 cell lines without transfection were named as CON group.

Table 1 Clinical and histopathologic data of the osteosarcoma patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>Number of cases (percentage of NSBP1 positively staining cells [%])</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>52 (52.8±5.5 %)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;40</td>
<td>38 (56.8±5.5 %)</td>
<td>0.83</td>
</tr>
<tr>
<td>≥40</td>
<td>14 (51.0±5.5 %)</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>40 (55.7±3.6 %)</td>
<td>0.51</td>
</tr>
<tr>
<td>Female</td>
<td>12 (52.0±5.0 %)</td>
<td></td>
</tr>
<tr>
<td>Pathologic staging</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>6 (25.8±3.3 %)</td>
<td>0.03</td>
</tr>
<tr>
<td>II</td>
<td>31 (52.8±5.5 %)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>3 (68.1±3.5 %)</td>
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<tr>
<td>TNM staging</td>
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<tr>
<td>T1</td>
<td>12 (28.8±4.2 %)</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>1 (34.4±3.8 %)</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>5 (65.6±2.3 %)</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>35 (72.3±5.2 %)</td>
<td>0.01</td>
</tr>
<tr>
<td>Distant metastases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>34 (35±4.3 %)</td>
<td>0.04</td>
</tr>
<tr>
<td>Yes</td>
<td>6 (53.5±4.3 %)</td>
<td></td>
</tr>
</tbody>
</table>

Tissue microarray and IHC assay

Fifty-two osteosarcoma samples and their ANCT were examined by immunohistochemistry for the expression of HMGN5. Briefly, the tumour foci were selected for construction of the tissue microarrays and the tissue core biopsies were transferred to the recipient paraffin block at defined array positions. HMGN5 antibody (1:100 dilutions) was used for IHC assay of protein expression in tissue microarrays.
negative control was performed using a section without a primary antibody. Endogenous peroxidase was blocked by incubation with freshly prepared 0.3 % hydrogen peroxide with 0.1 % sodium azide for 15 min. Non-specific staining was blocked by incubation with 5 % bovine serum albumin at room temperature for 30 min. Tissue microarrays were incubated with biotinylated antibodies and horseradish peroxidase. Staining was developed with diaminobenzidine substrate and sections were counterstained with hematoxylin. The sections were observed and photographed with the optical microscope (Olympus, Tokyo, Japan). Using the Image-Pro-Plus 6.0 analytic system (IPP 6.0, Media Cybernetics, Inc.), the HMGN5 immunoreactive and non-immunoreactive cells with a nucleus were counted in three sections (with interval three sections) per patient in each group. A percentage was calculated by the immunostained/total neuronal ratio×100 %.

Western blot assay

The cells were extracted at indicated time using lysis buffer. Protease and phosphatase with Whole-cell extracts were prepared in RIPA buffer. Cell extracts were boiled for 10 min in loading buffer and then equal amount of cell extracts were separated on 6–5 % SDS-PAGE gels. Separated protein bands were transferred into polyvinylidene fluoride (PVDF) membranes and the membranes were blocked in 5 % skim milk powder. The primary antibodies against HMGN5, PCNA, PI3Kp85α and Akt, p-Akt, MMP-9, β-actin, cleaved PARP, cleaved caspase-3, LC3II/I, Beclin-1, p21 and cyclin B1 were diluted according to the instructions of antibodies and incubated overnight at 4°C. Subsequently, horseradish peroxidase-linked secondary antibodies were incubated at room temperature for 4 h at a dilution ratio of 1:1,000 according to the kit’s instruction. The membranes were washed with TBST for three times (5 min for each) and the immunoreactive bands were visualized using ECL-PLUS/Kit. The relative protein level was normalized to β-actin concentration.

Cell proliferation assay

Cell proliferation was analyzed with the CCK-8 assay. Briefly, cells infected with HMGN5 siRNA or control siRNA were incubated in 96-well plates at a density of 1×10^5 cells/well. Cells were treated with 10 μl CCK-8 dye at 0, 24, 48 and 72 h and then measured at 450 nm with enzyme immunoassay analyzer (Bio-Rad, American). The proliferation activity was calculated for each clone.

Wound-healing assay

After cells infected with HMGN5 siRNA or control siRNA for 24 h, transfected cells were plated in each well of a 12-well culture plate and allowed to grow to 80 % confluence. A wound was created using a 10-μl micropipette tip and the migration of cells towards the wound was monitored at 24 and 48 h.

Transwell invasion assay

Transwell filters were coated with matrigel on the upper surface of a polycarbonate membrane. After incubating at 37 °C for 30 min, the matrigel were solidified and served as the extracellular matrix for analysis of tumor cell invasion. Briefly, the cells were trypsinized with 0.25 % trypsin containing EDTA and then implanted with each transwell. Undergoing migration for 24 h, a cotton swab was used to remove the non-migrated cells in the upper chamber and then fixed with 100 % methanol, stained with hematoxylin, and mounted and dried at 80 °C for 30 min. The cell numbers were counted in five random fields of each chamber under the microscope.

Cell cycle analysis

For cell cycle analysis, cells were trypsinized, washed by PBS and fixed with 80 % cold ethanol overnight at 4°C. After PBS washing, the fixed cells were stained with PI in the presence of RNase A (10 μg/ml) for 30 min at room temperature in darkness. Subsequently, sample was filtered through a 50-μm nylon filter to obtain single-cell suspension. The cell cycle phase distribution was determined by analytical DNA flow cytometry. The percentage of cells in each phase of the cell cycle was analyzed using Modfit software (Verity Software House, Topsham, ME, USA) [14].

Statistical analysis

All data were reported as means ± standard deviation (SD) for at least three independent experiments. SPSS 13.0 was used for the statistical analysis. Kruskal–Wallis H test and Chi-square test were used to analyze the expression rate in all groups. 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. Statistical significance was set at $P<0.05$. 

[Springer]
Results

The expression of HMGN5 protein in human osteosarcoma and the correlation of HMGN5 protein expression with the clinical and pathologic characteristics

HMGN5 staining in osteosarcoma and ANCT was both detected in the nucleus by using IHC assay. Furthermore, the mean positive cell incidence of HMGN5 protein staining was 17.0±3.9 % in ANCTs, as compared with 53.5±4.3 % in the osteosarcoma tissues with a significant difference (t=2.08; P<0.01) (Fig. 1a). The relationship between HMGN5 expression and various clinical and pathologic features was further analyzed. As shown in Table 1, the positive cell incidence of HMGN5 protein staining was correlated with the clinical and pathologic characteristics of osteosarcoma, but no significant correlation was found between HMGN5 expression with age or gender (P=0.83; P=0.51). According to the pathologic staging and TNM staging as previously described [15], the group with stage II–III, T3–T4 and distant metastasis showed more positive expression of HMGN5 protein (P=0.03; P=0.01; P=0.04).

Effect of HMGN5 knockdown on osteosarcoma cell proliferation

To confirm the role of HMGN5, we adopted specific gene interference technique. siRNAs targeting at HMGN5 were successfully transfected into U2-OS and SaO2 cells, and dramatically decreased protein expressions of HMGN5 were verified in the transfected cells by Western blot analysis (Fig. 1b).

Deregulated cell proliferation is a hallmark of cancer [16]. In order to test the effect of HMGN5 knockdown on U2-OS cell proliferative activities, the CCK-8 assay was adopted for cells after HMGN5 knockdown. It was indicated that HMGN5 significantly reduced the proliferative activities of U2-OS and SaO2 cells in a time-dependent manner.
and SaO2 cells growth, we investigated the proliferative activities by WST. As a result, knockdown of HMGN5 could significantly reduce the proliferative activities of U2-OS and SaO2 cells compared with NC and CON groups (Fig. 1c).

In addition, PCNA, a nuclear protein, is a marker for cell proliferation and PI3K/AKT is required for regulating cell proliferation. In order to determine whether knockdown of HMGN5 suppressed the endogenous expression of PI3K/AKT and PCNA through translational repression, Western blot assays were performed at 48 h recovery to measure the exogenous expression of PCNA, PI3Kp85 and p-AKT. As a result, the expressions of PCNA, PI3Kp85 and p-AKT protein were significantly decreased in siHMGN5 group compared with CON and siCON groups (**P<0.01) (Fig. 1b), suggesting that knockdown of HMGN5 might inhibit osteosarcoma cell proliferation through down-regulation of PI3K/AKT and PCNA expression.

Effect of HMGN5 knockdown on osteosarcoma cycle distribution

The cycle distribution of U2-OS and SaO2 cells was also analyzed. As shown in Fig. 2a, cell cycle was arrested in G0/G1 phase in siHMGN5 group compared with CON and siCON groups, although the S phase fraction was similar among these groups. Subsequently, p21 (a negative regulator) and cyclin B1 (a positive regulator), two regulators of progression from G1 to S, were assessed with Western blot [17]. The results showed that knockdown of HMGN5 only led to the decreased level of cyclin B1, but no significant changes in the levels of p21 (Fig. 2b), suggesting that HMGN5 might modulate cell cycle through regulation of cyclin B1.

Effect of HMGN5 knockdown on osteosarcoma cell invasion and metastasis

To determine the effect of HMGN5 knockdown on osteosarcoma cell invasion and metastasis, transwell assay and wound-healing assay was carried out. As shown in Fig. 3a, the migration of U2-OS and SaO2 cells in siHMGN5 group was markedly lower than in CON and siCON groups. However, there were no significant differences between the CON and siCON groups. Furthermore, a transwell assay was performed to determine the ability of cells to invade a matrix barrier and the representative micrographs of transwell filters are presented in Fig. 3b. The invasive cell count demonstrated that invasive potential was significantly reduced in the siHMGN5 group relative to the CON and siCON groups (Table 2). In addition, Western blot assay was performed to investigate the effect of HMGN5 knockdown on the endogenous expression of MMP-9 protein since MMP-9 was involved in tumor invasion and metastasis in a previous research [11]. As shown in Fig. 3c, there was a significant reduction in MMP-9 expression in siHMGN5 group compared with CON and siCON groups (*P<0.05). These data demonstrated that knockdown of HMGN5 might inhibit osteosarcoma invasion and metastasis through down-regulation of MMP-9 expression.

HMGN5 knockdown increases apoptosis and sensitivity to chemotherapy

To determine whether knockdown of HMGN5 affected osteosarcoma cell apoptosis under low serum treatment and doxorubicin conditions, cell viability was first performed. As shown in Fig. 4a, after (0%, 0.1%, 1%) low serum treatment for 24 h, there was no significant difference in cell viability among siHMGN5, CON and siCON groups. Interestingly, after (0.1, 1 and 10μg/ml) doxorubicin treatment for 24 h, knockdown of HMGN5 in U2-OS and SaO2 cells rendered them significantly more sensitive to doxorubicin-induced cell injury (Fig. 4b). Moreover, cell death could be categorized into necrotic, apoptotic and autophagic [18, 19]. Trypan blue exclusion assay revealed that the incidence of cell necrosis in all groups was less than 5% after 10μg/ml doxorubicin treatment (Fig. 4c), suggesting that the doxorubicin-induced cell injury was a result of apoptosis or autophagy. Subsequently, to distinguish apoptosis or autophagy, the cleavage of PARP and cleaved caspase-3 served as markers of cells undergoing apoptosis and LC3II/I and Beclin-1 served as markers of cells undergoing autophagic cell death. As shown in Fig. 4d, the levels of cleavage of PARP and cleaved caspase-3 in siHMGN5 group was markedly higher than that in CON and siCON groups after exposure to 10μg/ml doxorubicin for 24 h, but no significant changes were found in the levels of LC3II/I and Beclin-1 expressions among these groups. The results demonstrated that knockdown of HMGN5 increased sensitivity to doxorubicin-induced cell injury through activating apoptotic cell death.

Discussion

HMGN5, also named nucleosome binding protein 1, is a nuclear protein which contains three important functional domains, the nuclear localization signal, the NBD, and a conserved motif in the C-terminal domain [20]. HMGN5 binds to nucleosomes, unfolds chromatin via the NBD and affects transcription. HMGN5 is widely expressed in various human tissues and associated with cancer development, progression, and metastasis in human [11, 12] and mice [21]. Increased expression of HMGN5 protein was observed prostate cancer [7] and squamous cell carcinoma in human [8], adenocarcinoma in mice [9] and breast cancer cell line [10], suggesting a role for HMGN5 in tumorigenesis. Additionally, HMGN5 may also contribute to the differentiation by
inducing chromatin structure changes in mouse embryonic stem cells [6]. Thus, HMGN5 targeting is a potential therapeutic strategy against cancer development, progression, and metastasis.

Osteosarcoma is the most common bone cancer in children and treatment failures are common due to tumor progression, multi-drug resistant, and distant metastasis. Therefore, the underlying molecular mechanisms need to be further explored. HMGN5 plays a critical factor in the development of osteosarcoma, and it offers a novel target for improving cancer therapy [13]. In our study, HMGN5 expression was significantly elevated in the nucleus of osteosarcoma tissues compared with the ANCT, suggesting that the carcinogenesis might be associated with the aberrant expression of HMGN5 in the nucleus of osteosarcoma tissues. The relationship between HMGN5 expression and various clinical and pathologic features was analyzed. Importantly, it was found that HMGN5 expression did correlated with the clinicopathologic characteristics of osteosarcoma patients including pathological grade, TNM staging and distant metastases. To confirm our hypothesis, we further applied RNAi to efficiently downregulate HMGN5 expression in U2-OS and SaO2 osteosarcoma cancer cell lines and HMGN5 knockdown greatly inhibited osteosarcoma cells growth in vitro. Knockdown of HMGN5 also inhibited the invasive and metastasis potential of U2-OS and SaO2 cells, indicating that HMGN5 might play an
important role in the development and progression of osteosarcoma, and represent a potential therapeutic target for treatment of cancer.

Moreover, HMGN5-regulated downstream molecular signaling was further explored in osteosarcoma cells. It is reported that PI3K/AKT pathway is the predominant signaling cascade required for osteosarcoma growth and metastasis [22]. Consistently, our study also showed that knockdown of HMGN5 decreased the expression of PI3K/AKT, and inhibited the proliferative activities and metastatic potential of osteosarcoma cells, indicating that HMGN5 might involve in tumor proliferation and metastasis through PI3K/AKT signaling pathway. In addition, proliferative cellular nuclear antigen (PCNA) is highly expressed in S-phase and required for maintaining cell proliferation in proliferating cells and HMGN5 could inhibit tumor invasion and metastasis in a previous research through down-regulation of MMP-9 [11]. Consistent with previous two reports, our study result also showed a marked decrease of PCNA and MMP-9 expression, suggesting that HMGN5 might down-regulate PCNA and MMP-9 expression via the PI3K/AKT signaling pathway.

We also found that knockdown of HMGN5 led to cell cycle arrest in G0/G1 phase. To further confirm that HMGN5 modulated the cell cycle progression of osteosarcoma cells, we examined the levels of p21 and cyclin B1, which were necessary for the crucial checkpoints of G1/S transition [17, 23]. As expected, HMGN5 knockdown induced a rapid decrease of cyclin B1 expression. Our results suggested that HMGN5 might modulate cell cycle through the regulation of cyclin B1, which was in accordance with a previous result [11]. The solid tumors in early stages possess poorly developed blood

**Table 2** The number of invasive cells in CON, siCON and siHMGN5 groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of the invasive cells</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>U2-OS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>60±12</td>
<td>0.01</td>
</tr>
<tr>
<td>siCON</td>
<td>59±9</td>
<td></td>
</tr>
<tr>
<td>siHMGN5</td>
<td>43±11</td>
<td></td>
</tr>
<tr>
<td>SaO2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>25±4.2</td>
<td>0.03</td>
</tr>
<tr>
<td>siCON</td>
<td>27±5.2</td>
<td></td>
</tr>
<tr>
<td>siHMGN5</td>
<td>20±3.6</td>
<td></td>
</tr>
</tbody>
</table>
vessels, leading to the deprivations of nutrients in the central region \[24\], and doxorubicin is commonly used anticancer drugs in osteosarcoma \[25\]. Therefore, we next investigated whether HMGN5 knockdown increased susceptibility to apoptosis induced by serum deprivation or doxorubicin. Importantly, data from the present study revealed that knockdown of HMGN5 rendered them significantly more sensitive to doxorubicin-induced cell injury through activating apoptotic cell death.

Fig. 4 Inhibition of HMGN5 expression increased osteosarcoma cells sensitivity to doxorubicin-induced cell injury in vitro. a CCK-8 assay for cells after doxorubicin (Dox) or low serum treatment. After (0 %, 0.1 %, 1 %) low serum treatment for 24 h, a distinct decrease of cell viability was detected but no significant among siHMGN5, CON and siCON groups. However, after (0.1, 1 and 10 μg/ml) doxorubicin treatment for 24 h, cell viability in siHMGN5 was a lower than that in CON and siCON groups. b Trypan blue exclusion assay for osteosarcoma cells after doxorubicin treatment. The results revealed that the incidence of cell necrosis was always less than 5 % after 10 μg/ml doxorubicin treatment. c Western blot for cleavage of PARP, cleaved caspase-3, and LC3II/I and Beclin-1 after 10 μg/ml Dox treatment for 24 h. Osteosarcoma cells were treated with 10 μg/ml Dox for 24 h, and then the proteins were analyzed by Western blot assay. The result indicated that the levels of cleavage of PARP and cleaved caspase-3 in siHMGN5 group were markedly higher than those in CON and siCON groups, but no significant changes in the levels of LC3II/I and Beclin-1 expressions were found among these groups.
To our knowledge, this is the first report investigating the role and clinical significance of HMGN5 in osteosarcoma, but the use of osteosarcoma cells from two cell lines just provides very limited evidence. Further research using more cell lines, xenograft model and primary tumor in vivo is indispensable to confirm our hypothesis. In conclusion, our investigation reveals that HMGN5 is highly expressed in the nucleus of osteosarcoma tissues, and implicated in many aspects of tumor progression, including cell growth, cell cycle regulation, invasion, and migration. Intervention with HMGN5 expression and function by agents such as siRNA may have potential application in the treatment of osteosarcoma, and provide a promising therapeutic target for osteosarcoma.

Conflicts of interest No conflict of interest to disclose.

References
