# *Let-7* repression leads to HMGA2 overexpression in uterine leiomyosarcoma

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## Abstract

Overexpression of HMGA2 is common in uterine leiomyomas (ULM). The expression of *HMGA2* in its malignant counterpart – uterine leiomyosarcomas (ULMS) remains undetermined. Recently it has been shown that repression of *HMGA2* by microRNA *let-7s* is a critical molecular regulatory mechanism associated with tumour growth in many tumours and cell types, including leiomyomas. To test whether *HMGA2* and *let-7s* play a role in ULMS, we examined the levels of endogenous *HMGA2* and *let-7* expression and found a significant correlation between these two molecules in a case-matched cohort of human ULMS. We found that overexpression of *HMGA2* and *let-7*-mediated *HMGA2* repression is a relevant molecular alteration in ULMS. Disrupting the control of *HMGA2* and *let-7* pairs promotes ULMS cell growth *in vitro*.

## Introduction

Uterine leiomyosarcomas (ULMS) are rare neoplasms, representing 1 in 200 to 800 smooth muscle tumours or ~1% of all malignancies of the uterus [1]. Although the reported 5-year ULMS survival rates are variable, these tumours are clinically aggressive and have a high risk of recurrence and overall poor prognosis [2]. The cause or the molecular basis of human ULMS is largely unknown. While it was believed that most ULMS could arise *de novo*, some reports suggest that ULMS was progressive from pre-existing uterine leiomyomas (ULM) [3, 4]. However, the conclusive evidence documenting the transformation of ULM to ULMS is lacking in human beings and it is thought that the incidence of transformation is <0.1% [5].

HMGA2, a high-mobility-group AT-hook (HMGA) protein, is considered to function as an oncogene strongly associated with many malignant epithelial and mesenchymal neoplasms [6]. It has three AT-hook DNA binding domains, through which *HMGA2* binds to AT-rich sequences in the minor groove of the DNA helix. *HMGA2* is expressed in embryonic tissue but not in most adult tissues [7, 8]. *HMGA2* is overexpressed in ULM due to chromosomal 12q15 changes [9–12]. Overexpression of *HMGA2* in ULMS has been suggested, but not fully investigated [4, 13]. *HMGA2* is an important regulator of cell growth, differentiation, apoptosis and transformation [14]. New evidence suggest that dysregulation of microRNAs (miRNAs) may play a central role in tumour development [15]. Members of the let-7 miRNA family function as tumour suppressors through specific repression of its target gene, particularly of *HMGA2* expression in some tumour cells both *in vivo* and *in vitro* [4, 16, 17]. The biological importance of molecular pairing of *HMGA2::let-7* was further illustrated by the demonstration that repression of *HMGA2* by let-7s impairs tumour cell proliferation in many different tumour types, including ULM [4, 16–19]. It would be of great interest to characterize whether overexpression of *HMGA2* and disruption of the *HMGA2::let-7* pairs contributes to the aggressive growth behaviour of ULMS.

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In this study, we examined the levels of endogenous *HMGA2* and *let-7* expression and analysed a potential correlation between these two molecules in a case-matched cohort of human ULMS. We demonstrate that overexpression of *HMGA2* and *let-7*-mediated *HMGA2* repression is important for molecular changes in ULMS. Disrupting the *HMGA2::let-7* pairs promote ULMS cell growth *in vitro*.

# Materials and methods

## Patient and tissue samples

A total of 35 hysterectomies for ULMS were collected in this study. The mean ages at hysterectomies were 59.9 years old (ranging from 38 to 83 years old). Tumour sizes were ranged from 2 to 22 cm with mean tumour size of 11.6 cm. Of all 35 cases, 23 were solitary and 12 coexisted with leiomyomas (Table 1).

High-density tissue microarray (TMA) was prepared from formalin fixed and paraffin-embedded (FFPE) tissue cores (0.6 mm) in ULMS ($n = 30$) and matched myometrium ($n = 30$) in triplicate. Fresh frozen tissues from ULMS and matched myometrium were collected in five cases for RT-PCR and Western blot analysis (Table 1).

Three uterine leiomyosarcoma cell lines were used for the study including: SK-LMS-1, SK-UT-1 and SK-UT-1b.

The study was approved by the NYU Medical Center institutional review board.

## *Let-7* mimic and inhibitor

Mature double stranded miRNAs of *let-7* and *let-7* inhibitors were purchased from Dharmacon, Inc. (Lafayette, CO, USA). All experiments were controlled using a non-functional double-stranded random 22 nt RNA (Block-iT, Invitrogen, Carlsbad, CA, USA).

## Primers and antibodies

Primers from *HMGA2* and its alternative spliced transcripts were reported previously [19]. Primers for mature *let-7* family (*a–i*) were purchased from Ambion, Inc. (Austin, TX, USA). Antibodies includes HMGA2 (provided by Dr. Masashi Norita and Biocheck, CA, USA), Ki-67, ER$\alpha$ and PR-A (Ventana Medical System, Inc., Tucson, AZ, USA), and P53 (Neomarkers, CA, USA).

## *Let-7c* miRNAs *in situ* hybridization

The hybridization system and probes, miRCURY LNA, *Let-7*c, and *U6*, were purchased from Exiqon (Vedbaek, Denmark). The detailed procedure for *in situ* hybridization was followed as per manufacturer's protocol [20]. In brief, 4-$\mu$m TMA slides were prepared. Following deparaffinization and deproteinization, the slides were pre-hybridized with 1$\times$ hybridization buffer without probe. The hybridization was carried out overnight in a 1$\times$ hybridization buffer (30–70 $\mu$l) with pre-denatured miRCURY LNA, *let-7*c or *U6* probes. After washing, the slides were blocked and incubated with

AP conjugated anti-DIG Fab fragments (1:1500, Roche, Indianapolis, IN, USA) and visualized for colour detection.

## qRT-PCR

For the detection of mature miRNAs, *mir*Vana qRT-PCR primers and the *mir*Vana qRT-PCR Detection Kits (Ambion, Inc.) were used and optimized according to the abundance of miRNAs in the samples. In brief, 50 to 100 ng of total RNA were reverse transcribed with specific miRNA primers. A total of 15–30 cycles were performed for quantitation. Primers for the common domain of *HMGA2*, the dominant transcript (*HMGA2*a), and the cryptic *HMGA2* transcripts were described [19]. The abundances of cDNA products were detected by qRT-PCR and were normalized by the internal control products of U6 and $\alpha$-Actin.

## Immunohistochemistry

The TMA blocks from FFPE tissues were sectioned at 4 microns. After deparaffinization and antigen retrieval, all immunohistochemical staining was performed on a Ventana Nexus automated system.

## Western blot analysis

Fresh frozen tissue or culture cell samples were homogenized at 4°C in a protein lysis buffer (0.5 g tissue in 1–2 ml). Identical amounts of total proteins from each sample were separated through a 12% SDS-PAGE gel and then transferred to a PVDF membrane (Perkin Elmer Life Scientific Inc.). Development of the immunoblot with antisera against HMGA2 and negative control HMGA2 blocking peptide (provided by Dr. Masashi Norita and Santa Cruz Biotechnology, Inc., CA, USA) was tested and a single specific HMGA2 band at 25 kD was detected, as previously described.

## Cell culture and miRNAs transfection

LMS cell lines were maintained in Dulbecco's modified Eagle's medium (Invitrogen) containing 10% foetal bovine serum (Gemini, Calabasas, CA, USA) in 37°C incubators with 5% CO$_2$ until the cells reached 30–40% confluence. Prior to transfection, cells were placed in standard media without antibiotics for 24 hrs. As per manufacturers' protocol, transfection was performed with the Lipofectamine system with miRNAs concentrations of 20–60 pmol/well in either 6- or 24-well plates. To estimate transfection efficiency, cotransfection with the block-iT fluorescent double-stranded random 22mer RNA from Invitrogen was performed. The FITC fluorescence was visualized by $l_{ex} = 494$ nm and $l_{em} = 519$ nm to assess the percentage of cells that were successfully transfected. Cells receiving only the tagged random sequence double-strand 22mer were used as non-specific references at all data points. Following transfection, cells were harvested and analysed at the indicated times.

## Cellular proliferation assay

PC3 and LNCaP cell lines of control (with pBabe) and of tests (stable over-expression of *HMGA2*a with and without *let-7* LCS) were passed in 24-well plates in triplicate at densities of $5 \times 10^3$ cells/well for LNCaP and $1 \times 10^4$

**Table 1** HMGA2 and let-7s expression in 35 uterine leiomyosarcomas

| Case no. | Age (years) | Size (cm) | HMGA2 (IHC) | HMGA2 (RT) | Let-7c (ISH) | Let-7 (c, d, f-2) | Ki-67 index (%) |
|----------|-------------|-----------|-------------|------------|--------------|-------------------|-----------------|
| ULMS1 | 43 | 5 | 0 | | 0 | | 30 |
| ULMS2 | 57 | 4 | 0 | | −2 | | 15 |
| ULMS3 | 55 | 4 | 0 | | 1 | | 50 |
| ULMS4 | 53 | 10.5 | 0 | | 0 | | 60 |
| ULMS5 | 54 | 11# | 0 | | −1 | | 50 |
| ULMS6 | 79 | 30 | 0 | | −1 | | 25 |
| ULMS7 | 83 | 13 | 2+ | | −1 | | 10 |
| ULMS8 | 38 | 17 | 0 | | 0 | | 25 |
| ULMS9 | 82 | 9 | 0 | | 0 | | 35 |
| ULMS10 | 69 | 8.5 | 3+ | | 1 | | 25 |
| ULMS11 | 85 | ?*** | 3+ | | −1 | | 25 |
| ULMS12 | 59 | 15 | 0 | | 0 | | 30 |
| ULMS13 | 74 | 5 | 0 | | 0 | | 5 |
| ULMS14 | 50 | 6.8 | 0 | | 0 | | 50 |
| ULMS15 | 52 | 22# | 3+ | | −2 | | 40 |
| ULMS16 | 60 | 12 | 2+ | | −1 | | 15 |
| ULMS17 | 55 | 9 | 0 | | −1 | | 30 |
| ULMS18 | 73 | 11 | 2+ | | −2 | | 60 |
| ULMS19 | 46 | ? | 0 | | 1 | | 20 |
| ULMS20 | 64 | ? | 3+ | | −1 | | 35 |
| ULMS21 | 64 | 8 | 0 | | 0 | | 30 |
| ULMS22 | 48 | 7 | 0 | | 0 | | 5 |
| ULMS23 | 63 | ? | 0 | | 1 | | |
| ULMS24 | 59 | 4.5 | 0 | | 1 | | 60 |
| ULMS25 | 55 | ? | 0 | | 0 | | 60 |
| ULMS26 | 43 | 20 | 3+ | | 0 | | 20 |
| ULMS27 | 57 | 12 | 4+ | | 0 | | 30 |
| ULMS28 | 49 | 14 | 3+ | | −1 | | 50 |
| ULMS29 | 74 | ? | 3+ | | −1 | | 40 |
| ULMS30 | 60 | 2 | 0 | | | | |
| ULMS31 | 55 | 16 | | 3 | | 1, 0, 1 | |
| ULMS32 | 63 | 17 | 2* | 2 | | 2, 0, 1 | |
| ULMS33 | 51 | 20 | | 3 | | 3, 1, 3 | |
| ULMS34 | 53 | 21** | 2* | 2 | | 1, 0, 1 | |

Continued

**Table 1** Continued

| Case no. | Age (years) | Size (cm) | HMGA2 (IHC) | *HMGA2* (RT) | *Let-7c* (ISH) | *Let-7* (*c, d, f-2*) | Ki-67 index (%) |
|---|---|---|---|---|---|---|---|
| ULMS35 | 73 | 12 | | 4 | | 2, 1, 1 | |
| Mean | 59.94 | 11.62 | 1.13 | 2.50 | −0.35 | − | 33.21 |
| Sem | 2.00 | 1.07 | 0.24 | 0.18 | 0.15 | − | 3.06 |

*Western blot results.
**Karyotype: t(12; 14) and tri(12) (see Fig. 2); IHC: immunohistochemistry; ISH: *in situ* hybridization.
***: Tumour size is not documented in pathological report.
#: Tumours with extrauterine extension.

for PC3 cells. Cells were subsequently transfected with control RNA (non-function, Invitrogen), *let-7*c, and/or *let-7* inhibitors (Dharmacon, Inc.) at a dose of 40 pmol/well. Cellular proliferation was counted at 24, 48, 72 and 96 hrs using the colorimetric WST-1 assay (Cell proliferation Reagent, Roche). Briefly, the cells were incubated with 10% WST-1 reagent in normal medium for 2 hrs. Aliquots (100 μl) were then transferred to 96-well plates and the samples were read in a spectrophotometric plate reader at 450 nm (FLUOstar OPTIMA, BMG Lab Technologies, Durham, NC, USA).

## Statistical analysis

Mean and standard errors were calculated for the quantitative values. Statistical significance was analysed by a paired t-test and $P$-value $<0.05$ was considered significant.

# Results

## HMGA2 and *let-7* expression in ULMS

To evaluate whether elevated HMGA2 expression is common in ULMS, we examined HMGA2 expression in 30 ULMS by immuno-histochemistry analysis. With internal negative controls of matched myometrium and staining done with two different sources of HMGA2 antibodies (see section 'Materials and methods'), we found up to 38% of ULMS (11/29) immunoreactive for HMGA2 (Fig. 1). We then examined HMGA2 expression at transcription and translational levels by RT-PCR and Western blot, respectively, in five randomly selected ULMS collected from fresh frozen tissues. With a negative (matched myometrium) and a positive (HMGA2 positive ULM) controls, a moderate to high level of *HMGA2* mRNA expression (32 cycles) was detected in all five cases (Fig. 2A). Of same five tumours, two of them were positive for HMGA2 by Western blot analysis (Table 1, Fig. 2A).

The karyotype analysis was carried out in the latter five cases (ULMS31–35). One (ULMS-34) of five ULMS had non-random chromosomal changes involving chromosome 12 (Fig. 2B). These non-random chromosome 12 changes were commonly seen in usual type ULM. Further analysis of this tumour revealed a high level of HMGA2 mRNA and protein (Fig. 2A). This tumour had all

the features of malignant uterine smooth muscle tumour, including tumour necrosis, cytological atypia, invasion and high mitotic counts (Fig. 2B). We speculated that overexpression of HMGA2 in ULMS-34 was contributed by chromosome 12 changes same as those seen in usual ULM.

The levels of *Let-7* expression in ULMS were low based on semi-quantitative RT-PCR analysis in 5 ULMS (Fig. 2A). Among five let-7 family members (*let-7a, b, c, e, f-2*) examined, *let-7c* transcript was detectable in all ULMS. We therefore selected *let-7c* to analyse its expression in 30 ULMS by TMA based *in situ* hybridization (see section 'Materials and methods'). We used U6 as tissue quality control (Fig.1B). The relative levels of let-7c were first normalized by U6 (let-7c/U6 × 100%), and the net changes of *let-7c* in ULMS against matched myometrium (ULMS myometrium) were further calculated. Overall, there were slightly net losses of *let-7c* in ULMS in comparison to matched myometrium (−0.35 ± 0.15). A case-matched analysis of HMGA2 and *let-7c* expression was performed. As illustrated in Fig. 1A, in those ULMS with positive immunoreactivity for HMGA2, net loss of *let-7c* expression was quite more common than in tumours negative for HMGA2. Correlation analysis showed an inverse association between HMGA2 and let-7c expression ($r = -0.39$) (Table 1, Fig. 1A). In those five cases with RT-PCR data, higher levels of *HMGA2* mRNA in ULMS-32 and -34 were accompanied by lower levels of *let-7s* (Fig. 2A).

Correlation analyses were performed between HMGA2 expression and tumour size, tumour markers of Ki-67, P53, ERα and PR-A. We found a weak positive correlation of HMGA2 expression and tumour sizes ($r = 0.35$). There was not significant correlation of HMGA2 with other immunomarkers.

## Translational regulation of *HMGA2* by *let-7s* in ULMS cell lines

To test whether endogenous let-7s play a major role in repression of *HMGA2* in ULMS, we selected three ULMS cell lines for the study. We first examined *HMGA2* and its cryptic transcript (isoforms) [12, 21] expression in these cell lines by RT-PCR analysis. Since *HMGA2a* and its cryptic transcripts can be induced in primary
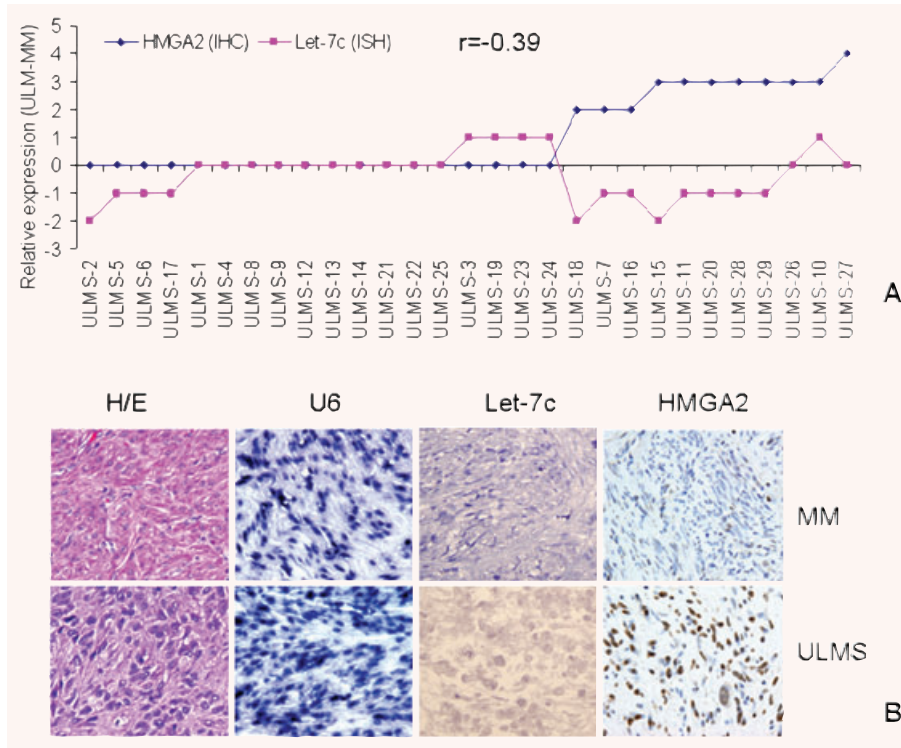
**Fig. 1** HMGA2 and *let-7c* expression in uterine leiomyosarcoma (ULMS) in a tissue microarray (TMA)-based analysis. A. Case-matched comparison of HMGA2 and *let-7c* expression in 30 ULMS. Net changes of *let-7c* (pink dots, detected by TMA-based tissue *in situ* hybridization with LNA *let-7c* probe (see section 'Materials and methods') and HMGA2 (blue dots, detected by immunohistochemistry) were scored in ULMS against matched myometrium. Among those ULMS with immunoreactivity for HMGA2, loss of *let-7c* was more frequent ($r = -0.39$). B. Photomicrographs illustrating examples of tissue cores from ULMS (bottom) and matched myometrium (MM, top) in haematoxylin and eosin staining, miRNA *in situ* hybridization for *U6* and *let-7c*, and immunohistochemistry for HMGA2.
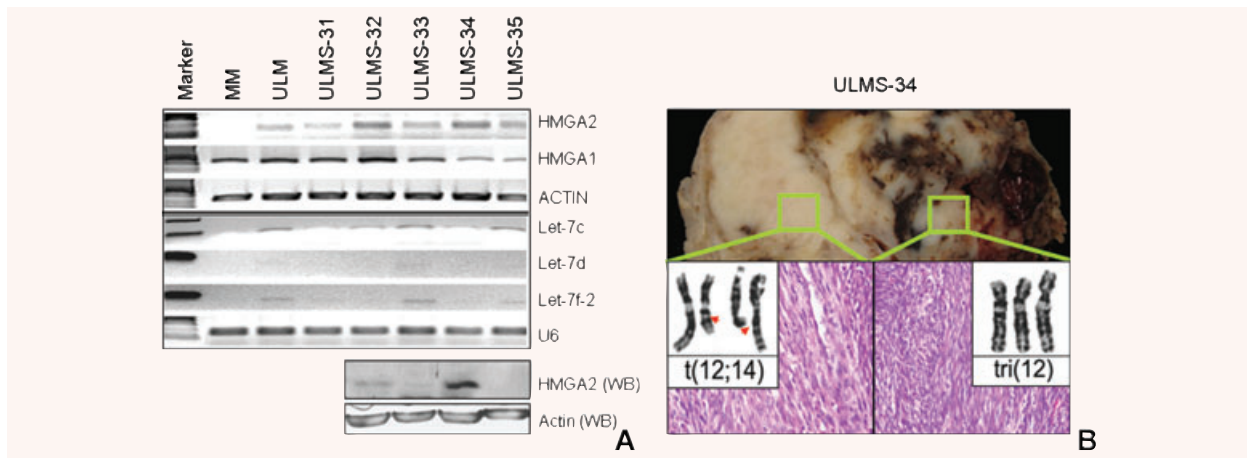


**Fig. 2** Semi-quantitative RT-PCR (upper panel) and Western blot (lower panel) analyses of *HMGA2* and some let-7 family members in fresh frozen tissue samples of five ULMS. (**A**) *HMGA2* mRNA was detectable in all 5 ULMS. One matched myometrium (MM) and one leiomyoma (ULM) were used as *HMGA2* negative and positive control, respectively. Actin was used as loading control. Among *let-7* members, *let-7c* was relatively higher than others in all ULMS. U6 was used as small RNA loading control. Western blot analysis of HMGA2 was examined in 4 ULMS (bottom). (**B**) Photographs illustrate gross appearance of a ULMS cross-section in case ULMS-34 and the corresponding karyotype (inserts with chromosome alterations) obtained in two different tumour regions.

cultured leiomyoma cells [12], we used cultured leiomyoma cells as a positive control. We found all ULMS cell lines had a high abundance of *HMGA2a* mRNA (Fig. 3A). In addition, *HMGA2* cryptic transcripts *c*, *d* and *f* showed moderate levels in these cell lines (Fig. 3A). In contrast, expression of *let-7s* was quite low in

comparison to normal myometrium and ULM based on a semi-quantitative analysis (Fig. 3B). *Let-7c* and *f* were the most abundant transcripts of *let-7* family in these cell lines.

To test the role of *let-7s* in repression of *HMGA2* after transcription and translation, we transfected the exogenous *let-7c* mimic
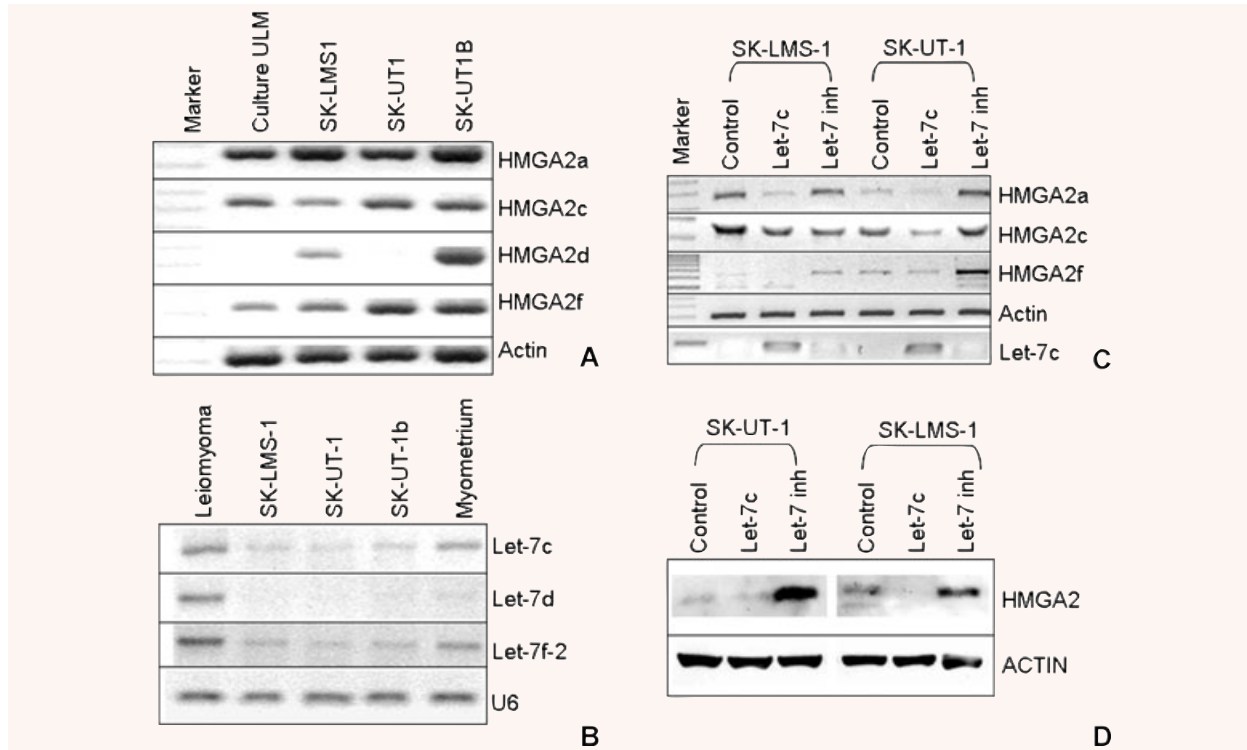
**Fig. 3** Molecular analysis of HMGA2 and *let-7* expression in three ULMS cell lines. (**A**) Semi-quantitative analysis of *HMGA2a* and its cryptic transcripts in ULMS cell lines of SK-LMS-1, SK-UT-1 and SK-UT-1b. Uterine leiomyoma (ULM) primary culture cells were used as a positive control. Actin was used as RNA loading control. (**B**) photonegative image showing relative expression of *let-7s* by RT-PCR in ULMS cell lines. One ULM and one myometrium were used as positive controls. U6 was used as loading control for small RNAs. (**C**) Transient transfection of exogenous *let-7c* and *let-7c* inhibitor and HMGA2 expression in LMS cell lines. Two LMS cell lines (SK-LMS1, SK-UT1) were treated with control miRNA (block-iT), *let-7c* and *let-7* inhibitor. *Let-7* levels in the treated cells were assessed by RT-PCR (bottom). Repression of *HMGA2* mRNA by *let-7c* was examined by semi-quantitative RT-PCR. Actin was used as control. (**D**) Western blot analysis of *HMGA2* gene product in LMS cell lines with different *let-7* levels, induced by transient transfection of control, *let-7c* or *let-7c* inhibitors.

and *let-7* inhibitor in all cell lines. In untreated cell lines (controls), low levels of endogenous *let-7s* seemed not enough to destabilize *HMGA2a* and other cryptic mRNA (Fig. 3A and B), but were sufficient to repress *HMGA2* translation (Fig. 3D, control lanes). When applying 40 *pmol* of exogenous *let-7c*, a significant reduction of *HMGA2a, c,* and *f* mRNAs (Fig. 3C) was observed, and a complete repression of HMGA2 protein was evident by a Western blot analysis (Fig. 3D, *let-7c* channel). When the cells treated with exogenous *let-7* inhibitor (which can completely block endogenous *let-7s*), up to fivefold increases of HMGA2 protein were observed in comparison to the untreated cells (Fig. 3D). The findings further supported that *HMGA2* repression by let-7s is specific regulatory mechanism for *HMGA2* expression in uterine leiomyosarcoma cells.

### Repression of HMGA2 inhibited ULMS cell growth

To test whether HMGA2 in ULMS cell lines plays a role in regulation of tumour growth rate, we examined the tumour cell proliferation by introducing exogenous *let-7* and *let-7* inhibitor, respec-

tively. The cells were treated in triplicate with Block-iT (control), *let-7c* and *let-7* inhibitor. Repression of *HMGA2* by *let-7c* and induction of *HMGA2* overexpression by *let-7* inhibitor were validated as shown in Fig. 3C and D, we counted for the tumour growth rates at days 1, 2 and 3 in all three ULMS cell lines (Fig. 4). Starting from day 2 (48 hrs), cells treated with *let-7c* had significantly lower rates of tumour cell growth than control ($P < 0.05$). The differential growth rates were even wider at day 3 (72 hrs). Particularly, the growth rates in tumour cells treated with *let-7* inhibitor were significantly higher than those in *let-7c* treated cells ($P < 0.05$), but at least two cell lines lower than those in control groups (Fig. 4). The later findings suggested that *let-7* mediated tumour growth in ULMS is not solely through repression of *HMGA2*, and other *let-7* target genes may be involved [22] (Fig. 4).

## Discussion

ULMS usually have very complicated cytogenetic alterations involving many different chromosomal regions. Cytogenetic
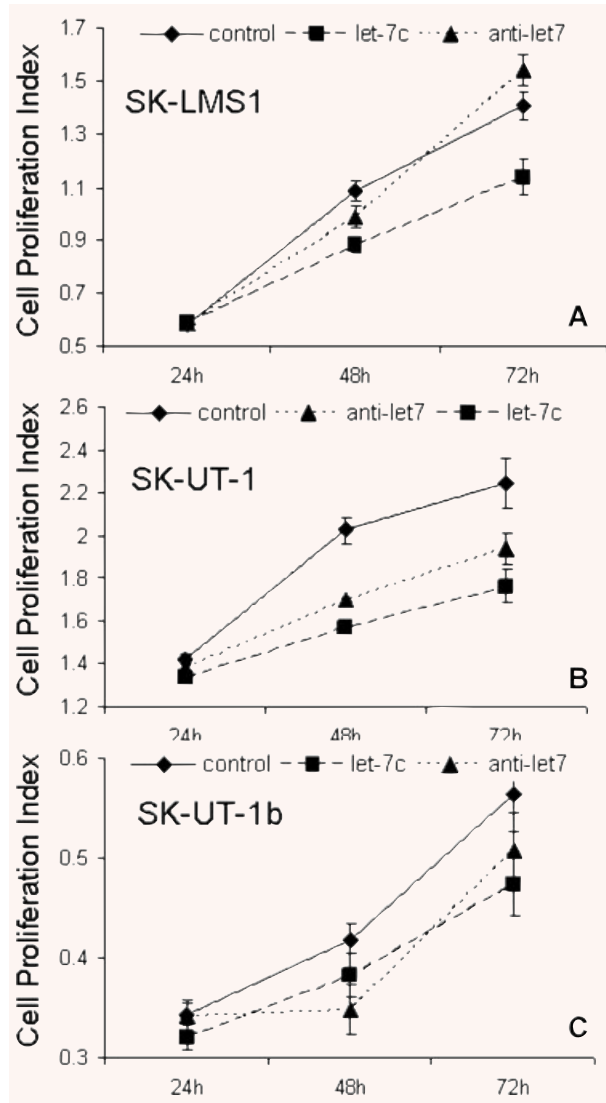
**Fig. 4** Anti-proliferative effects of *let-7* in ULMS cell lines SK-LMS-1 (**A**), SK-UT-1 (**B**) and SK-UT-1b (**C**). The cell lines were treated in three different conditions: (1) non-functional 20 nt RNA (block-iT, labelled as control, solid lines); (2) 40 *pmol* of exogenous *let-7c* (Dharmacon, Inc.) (long dash lines) and (3) exogenous *let-7* inhibitor (anti-*let-7*, short dash lines). Cell proliferation rates were calculated (*y*-axis) by WST-1 staining at 24, 48 and 72 hrs after transfection. Small t-bars were standard errors.

changes involving chromosomal 12q13–15 region in leiomyosarcomas had been occasionally reported through traditional karyotype [23] and comparative genomic hybridization (CGH) analysis [4], in which a gain of chromosome 12q15 region including HMGA2 was identified. Examination of HMGA2 expression in anatomic sites other than the uterus revealed overexpression of *HMGA2* mRNA in 57% of leiomyosarcomas by RT-PCR [13]. Examination of HMGA2 expression in ULMS has not been reported in the literature. This study is the first attempt to examine HMGA2

expression in ULMS. In this study, we observed that over one third of ULMS were immunoreactive for HMGA2. Furthermore, overexpression of *HMGA2* mRNA was observed in all native ULMS and ULMS cell lines in a small scale analysis. Given that only one of five ULMS showed chromosomal 12 changes, overexpression of *HMGA2* in ULMS seemed to be through an alternative mechanism.

The specific role of HMGA2 in tumourigenesis remains to be determined. Recent characterization of *let-7* regulation of *HMGA2* indicates that this molecular pair plays a critical role in the control of mitogenic activity in many different cell types and tumour cells [17, 18, 24, 25]. Particularly, it has been found that dysregulation of *let-7s* is associated with *HMGA2* expression in ULM and related to cell proliferation [17]. The later findings provided the evidence that this molecular pair is involved in benign uterine smooth muscle tumours. Further characterization of this molecular pair in its malignant counterpart ULMS may provide a new inside for our understanding of ULMS.

In this study, a seemly inverse association of HMGA2 protein and *let-7c* expression was observed. Although the global expression pattern of miRNA in ULMS remains to be determined, a loss of *let-7* expression was identified in a proportion of ULMS. Particularly, we illustrate that exogenous *let-7* can significantly reduce the tumour cell proliferation in leiomyosarcoma cell lines, largely through repression of *HMGA2* expression (Figs 3 and 4). Therefore, it is speculated that disrupting of *HMGA2* and *let-7* pairs in ULMS could be an important molecular change responsible for ULMS growth. In our future study, it will be important to compare whether overexpression of *HMGA2* and loss of *let-7* are associated with unfavourable clinical outcome and specific histological grade in a large cohort of ULMS cases.

Another interesting finding is that the karyotype characterized by specific chromosome 12 changes, commonly seen in benign ULM, was also identified in one ULMS in our study. Further analysis revealed an overexpression of *HMGA2* mRNA and protein in this tumour. The findings raise two questions:

(1) Can overexpression of *HMGA2* in ULMS be induced through specific chromosome changes involving chromosome 12, at least in a small proportion of tumours? A recent study of CGH in seven ULMS showed that one of the highest levels of genomic gains was in chromosome region 12q13–15 [4], where *HMGA2* is located. This study suggested that overexpression of *HMGA2* is commonly associated with increase genomic material in this region. Findings of two types of chromosome 12 changes involving different tumour regions in one leiomyosarcoma in this study apparently are not coincidence.

(2) Is there a molecular connection between benign and malignant uterine smooth muscle tumours to a non-random chromosome 12 changes? It is generally recognized that ULMS may develop independently from ULM, as it is (*i*) commonly a solitary mass of uterus, (*ii*) commonly in the peri- and post-menopausal women and (*iii*) composed of different genetic alterations as compared to leiomyomas. However, it is not uncommon that ULMS contain usual or atypical leiomyoma-like areas (LLA) [26]. The nature of LLA within ULMS has not yet been established. In the case with

chromosome 12 changes, the reason we selected two different regions for cytogenetic analysis was that this tumour consists of both histological malignant and LLA regions. It remains a challenge to characterize whether the chromosome 12q changes in ULMS arises from the possible LLA area or frankly from well-differentiated ULMS.

# Acknowledgement

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