Oncogene (2017) **36**, 3528–3540

www.nature.com/onc

ORIGINAL ARTICLE

# LncRNA HULC triggers autophagy via stabilizing Sirt1 and attenuates the chemosensitivity of HCC cells

H Xiong[1], Z Ni[1], J He[2], S Jiang[1], X Li[1], J He[1], W Gong[1], L Zheng[3], S Chen[1], B Li[1], N Zhang[1], X Lyu[1], G Huang[1], B Chen[4], Y Zhang[1] and F He[1]

Considerable evidences have shown that autophagy has an important role in tumor chemoresistance. However, it is still unknown whether the lncRNA HULC (highly upregulated in liver cancer) is involved in autophagy and chemoresistance of hepatocellular carcinoma (HCC). In this study, we for the first time demonstrated that treatment with antitumor reagents such as oxaliplatin, 5-fluorouracil and pirarubicin (THP) dramatically induced HULC expression and protective autophagy. Silencing of HULC sensitized HCC cells to the three antitumor reagents via inhibiting protective autophagy. Ectopic expression of HULC elicited the autophagy of HCC cells through stabilizing silent information regulator 1 (Sirt1) protein. The investigation for the corresponding mechanism by which HULC stabilized Sirt1 revealed that HULC upregulated ubiquitin-specific peptidase 22 (USP22), leading to the decrease of ubiquitin-mediated degradation of Sirt1 protein by removing the conjugated polyubiquitin chains from Sirt1. Moreover, we found that miR-6825-5p, miR-6845-5p and miR-6886-3p could decrease the level of USP22 protein by binding to the 3′-untranlated region of USP22 mRNA. All the three microRNAs (miRNAs) were downregulated by HULC, which resulted in the elevation of USP22. In addition, we showed that the level of HULC was positively correlated with that of Sirt1 protein in human HCC tissues. Collectively, our data reveals that the pathway 'HULC/USP22/Sirt1/ protective autophagy' attenuates the sensitivity of HCC cells to chemotherapeutic agents, suggesting that this pathway may be a novel target for developing sensitizing strategy to HCC chemotherapy.

*Oncogene* (2017) **36,** 3528–3540; doi:10.1038/onc.2016.521; published online 6 February 2017

## INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common human malignancies in the world, ranking as the second leading cause of cancer-related death.[1,2] Despite recent advances in the understanding of the molecular basis of HCC and new chemotherapeutic approaches, the mortality rate has declined only modestly because of chemoresistance of HCC. Recently, mounting evidences have revealed that protective autophagy is an important reason for the chemoresistance of cancer cells.[3,4] Autophagy, an conserved catabolic process, is responsible for disposing and recycling cellular proteins and damaged/excess organelles in response to starvation or cellular stresses such as chemotherapeutic agents.[5] Previous reports have shown that many chemotherapeutic drugs such as sorafenib, cisplatin and 5-fluorouracil (5-FU) can induce autophagy in HCC cells,[6,7] reducing the chemosensitivity and apoptosis, leading to the survival of cancer cells. Therefore, better understanding of the molecular events contributing to the protective autophagy and interfering them during chemotherapy would be beneficial to develop approaches to achieve sustained remissions of HCC patients.

Long noncoding RNAs (lncRNAs) are a class of transcripts longer than 200 nt with no or limited protein-coding potential. Mechanistically, lncRNAs exert their function by regulating gene expression in different levels, including epigenetic modulation, transcription (or post transcription) and translation regulation.[8] It has been demonstrated that lncRNAs are involved in physiological or pathological processes such as development, differentiation, apoptosis, autophagy, inflammation and cancer.[9] Lately, an increasing number of lncRNAs including HULC have been reported to have an important role in the carcinogenesis and development of HCC. HULC is the first identified lncRNA that is specifically overexpressed in human HCC tissues. It is upregulated by cAMP responsive element-binding protein,[10] and destabilized by insulin-like growth factors 2 mRNA-binding protein (IGFBP2).[11] Besides, hepatitis B virus X (HBX) protein can enhance HULC expression via activating cAMP responsive element-binding protein, leading to the proliferation of HCC cells.[12] Furthermore, HULC can promote HCC development by supporting abnormal lipid metabolism through activating acyl-CoA synthetase subunit ACSL1,[13] and contribute to HCC angiogenesis by upregulating sphingosine kinase 1 (SPHK1).[14] Collectively, these studies have clearly revealed that HULC has an important role in liver carcinogenesis and acts as an oncogenic lncRNA. However, to date, it is still unknown whether HULC can regulate the autophagy of HCC cells. Meanwhile, the role of HULC in response to chemotherapeutic reagents also remains unclear.

In the present study, we for the first time demonstrated that HULC can trigger autophagy in HCC cells via stabilizing Sirt1 protein. Moreover, we found that HULC decreased the expression of miR-6825-5p, miR6845-5p and miR6886-3p, which led to the upregulation of USP22 and Sirt1 proteins. In addition, silence of

[1]Department of Biochemistry and Molecular Biology, College of Basic Medical Sciences, Third Military Medical University, Chongqing, China; [2]Battalion 17 of Students, College of Preventive Medicine, Third Military Medical University, Chongqing, China; [3]Department of Hepatobiliary Surgery, Xinqiao Hospital, Third Military Medical University, Chongqing, China and [4]Laboratory Animal Center, Third Military Medical University, Chongqing, China. Correspondence: Professor F He or Dr Y Zhang or Professor B Chen, Department of Biochemistry and Molecular Biology, College of Basic Medical Sciences, Third Military Medical University, 30 Gaotanyan, Shapingba, Chongqing 400038, China.
E-mail: hefengtian06@aliyun.com or tmmubiozy@163.com or chenbb81@126.com
Received 10 June 2016; revised 21 November 2016; accepted 27 December 2016; published online 6 February 2017

HULC sensitized HCC cells to the antitumor reagents such as oxaliplatin, 5-FU and THP. Taken together, our data revealed that the pathway 'HULC/USP22/Sirt1/autophagy' attenuates the sensitivity of HCC cells to chemotherapeutic agents, suggesting that this pathway may be a novel target for developing new sensitizing strategy to HCC chemotherapy.

## RESULTS

### The level of Sirt1 protein parallels to that of HULC in a subset of human HCC tissues

Previous studies have demonstrated that Sirt1 is aberrantly upregulated in HCC tissues.[15] To validate this phenomenon, we investigated Sirt1 expression in 40 HCC tissues and the corresponding adjacent non-cancerous liver tissues. As shown in Figures 1a and b, the level of Sirt1 protein was elevated in 31 out of 40 HCC tissues compared with the corresponding non-cancerous liver tissues. However, the level of Sirt1 mRNA had no significant difference between the HCC and adjacent non-cancerous liver tissues (Figure 1c), which was further verified by the analysis of Sirt1 mRNA expression in large cohort of HCC patients using The Cancer Genome Atlas database (Supplementary Figure 1A). Moreover, we found that HULC was dramatically upregulated in HCC tissues compared with the corresponding non-cancerous liver tissues (Figure 1d). Intriguingly, the correlation analysis showed that the level of Sirt1 protein was paralleled to that of HULC in the HCC tissues (Figure 1e). Furthermore, we verified that there was a positive correlation between the levels of Sirt1 protein and HULC in 5 HCC cell lines and relatively normal hepatic cell line L02 (Supplementary Figures S1B–D). These results suggest that Sirt1 protein may be upregulated by HULC in HCC cells.

### HULC stabilizes Sirt1 protein

To determine whether Sirt1 could be elevated by HULC, HULC was overexpressed in HCC cells (Figures 2a and b). Interestingly, overexpression of HULC failed to upregulate Sirt1 mRNA (Figures 2c and d) but markedly elevated Sirt1 protein in HepG2 (Figure 2e) and Hep3B (Figure 2f) cells. To silence HULC, two siRNAs (si-HULC-1 and si-HULC-2) were used, and si-HULC-2 was more effective than si-HULC-1 in knockdown of Sirt1 (Supplementary Figure 2A). Hence, si-HULC-2 was chosen in the subsequent experiments. As shown in Supplementary Figure 2B, silence of HULC had little effect on Sirt1 mRNA, but significantly downregulated Sirt1 protein in HCC cells (Figure 2g). The above results indicated that HULC upregulates Sirt1 at the protein (but not mRNA) level. Subsequently, we investigated the effect of HULC on the protein stability of Sirt1. As shown in Figures 2h and i, overexpression of HULC could dramatically prolong the half-life of Sirt1 protein after added translation inhibitor cycloheximide (CHX). Conversely, silence of HULC remarkably decreased the half-life of Sirt1 protein (Figures 2j and k). These results indicated that HULC upregulates Sirt1 protein via enhancing its stability. Furthermore, the proteasome-specific inhibitor MG132 could protect Sirt1 protein from degradation in HULC-silenced HCC cells (Figures 2l and m), indicating that proteasome is involved in HULC-mediated inhibition of Sirt1 degradation.

### HULC promotes the deubiquitination of Sirt1 protein via inducing USP22

The above experiments have proved that proteasome is involved in Sirt1 degradation, we next explored the effect of HULC on Sirt1 ubiquitination. As shown in Figure 3a, overexpression of HULC decreased ubiquitination of Sirt1 and upregulated Sirt1 protein in HCC cells. Conversely, knockdown of HULC-enhanced ubiqitination of Sirt1 and downregualted Sirt1 protein (Figure 3b). Previous reports have demonstrated that Sirt1 ubiquitination is controlled by several proteins including MDM2,[16] USP22[17] and JNK.[18] Hence, we investigated which one of the three proteins is involved in the HULC-mediated deubiquitination of Sirt1. As shown in Figures 3c–e, HULC could significantly elevated USP22 protein in HCC cells. Silencing of HULC led to a marked decrease of USP22 protein (Figure 3f and g). To ascertain whether USP22 is involved in HULC-mediated Sirt1 induction, we firstly synthesized three specific siRNAs targeting USP22. Then we verified that all the three siRNAs could inhibit USP22 protein level in HCC cells (Supplementary Figures 2C and D) and the first siRNA (si-USP22-1) was chosen in the subsequent experiments. Secondly, we found that knockdown of USP22 dramatically attenuated HULC-induced Sirt1 protein level (Figure 3h) and Sirt1 deubiquitination (Figure 3i). Moreover, co-immunoprecipitation assays showed that USP22 formed a complex with Sirt1 (Figure 3j), which was consistent with previous study that USP22 can directly bind to Sirt1 and stabilize it.[17] Overexpression of HULC enhanced the binding between USP22 and Sirt1 via upregulating USP22 (Figure 3k). In addition, the co-localization between USP22 and Sirt1 was revealed by double immunofluorescent staining (Figure 3l), indicating that there is a possible interaction between the two molecules. Collectively, these results revealed that HULC promoted USP22 expression and its interaction with Sirt1, which removed the conjugated polyubiquitin chains from Sirt1 and enhanced the stabilization of Sirt1 protein.

### miR-6825-5p, miR-6845-5p and miR-6886-3p target and suppress USP22

To explore how HULC induces USP22 expression, USP22 mRNA level and its protein stability were detected after HULC overexpression. Interestingly, overexpression of HULC did not significantly change the level of USP22 mRNA (Supplementary Figures 3A and B) and the stability of USP22 protein (Supplementary Figures 3C and D) in HCC cells, indicating that neither transcriptional nor post-translational regulation of USP22 is involved in HULC-mediated USP22 induction. Subsequently, we investigated whether USP22 is regulated by microRNA (miRNA) at translational level. As shown in Figure 4a, miRNA microarray was performed after HULC overexpression, and five downregulated miRNAs (miR-4646-3p, miR-6751-3p, miR-6825-5p, miR-6845-5p and miR-6886-3p) were picked out by setting threshold values ($FC \leqslant -2$, $P \leqslant 0.05$, $n = 3$). Bioinformatics prediction with TargetScan and miRDB showed that miR-6825-5p and miR-6845-5p may potentially target USP22 3′-untranslated region (3′-UTR), and the potential binding sites were shown in Figure 4b. Interestingly, western blot analysis showed that miR-6886-3p could also dramatically decreased the protein levels of USP22 and Sirt1 in addition to the predicted miR-6825-5p and miR-6845-5p (Figures 4c-e). The potential binding site of miR-6886-3p at USP22 3′-UTR was shown in Figure 4b. Conversely, the inhibitors of the three miRNAs increased the protein levels of USP22 and Sirt1 (Figures 4f and g). Luciferase reporter assay revealed that the three miRNAs could directly target USP22 3′-UTR (Figure 4h). Collectively, these findings indicated that miR-6825-5p, miR-6845-5p and miR-6886-3p can target and suppress USP22.

### HULC inhibits the expression and activity of miR-6825-5p, miR-6845-5p and miR-6886-3p

As shown in Figures 5a and b, overexpression of HULC decreased the levels of miR-6825-5p, miR-6845-5p and miR-6886-3p in HCC cells. Conversely, knockdown of HULC increased the expression of the three miRNAs (Figures 5c and d). Luciferase reporter assays showed that ectopic expression of HULC increased the luciferase activity of USP22 3′-UTR (Figure 5e), and silence of HULC decreased the luciferase activity of USP22 3′-UTR (Figure 5f). Moreover, the HULC-induced luciferase activity of USP22 3′-UTR

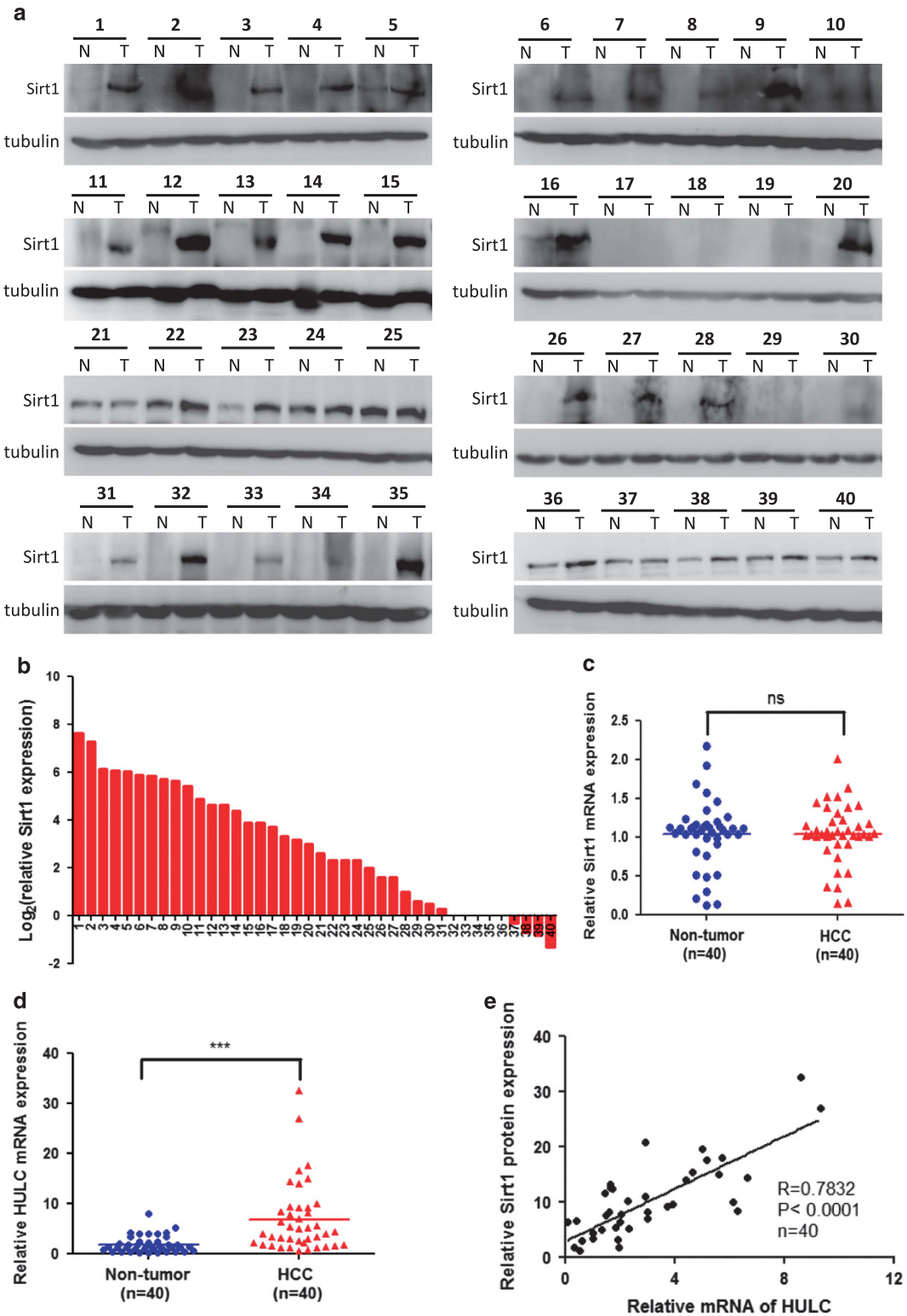


**Figure 1.** The level of Sirt1 protein parallels to that of HULC in a subset of human HCC tissues. (**a**) Western blot analysis of the expression of Sirt1 protein in 40 HCC tissues and the corresponding adjacent non-cancerous liver specimens, taking tubulin as a loading control. N, non-cancerous liver tissue; T, tumor tissue. (**b**) The quantitation of the western blot results in (**a**) using quantity-one software. The relative value of Sirt1 protein level was transformed to $Log_2$ (T/N). The numbers of the HCC patients were rearranged according to Sirt1 relative level. (**c**, **d**) qRT-PCR analysis for the levels of Sirt1 mRNA (**c**) and HULC (**d**) in the HCC and adjacent non-cancerous liver tissues, taking β-actin mRNA as a control. Statistical analysis was determined by paired Student's *t*-test. (ns, no significance; $^{***}P < 0.001$). (**e**) The correlation between the levels of HULC and Sirt1 protein in 40 HCC tissues. The statistical analysis was performed using Pearson's correlation coefficient ($R = 0.7832$, $P < 0.0001$). qRT-PCR, quantitative real-time PCR.

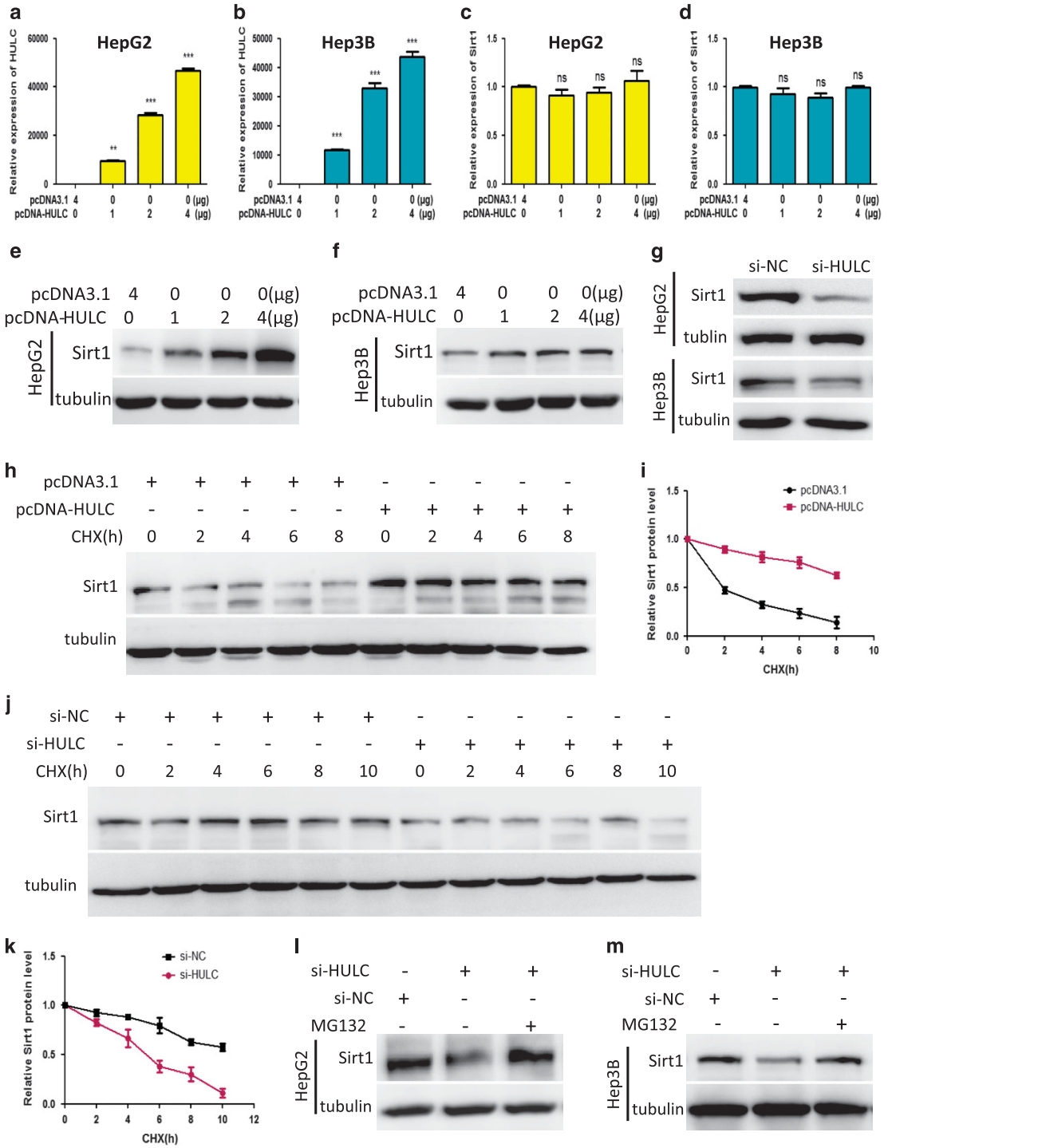


**Figure 2.** HULC upregulates Sirt1 protein via enhancing its stability. (**a**–**d**) HepG2 (**a**, **c**) and Hep3B (**b**, **d**) cells were transfected with control (pcDNA3.1) or various concentrations of pcDNA-HULC plasmid for 24 h. Then the levels of HULC (**a**, **b**) and Sirt1 mRNA (**c**, **d**) were determined by qRT-PCR, taking β-actin mRNA as a control. (**e**, **f**) The cells were treated as in **a**–**d**, and then the level of Sirt1 protein was examined by western blot. (**g**) The HCC cells were transfected with si-HULC or negative control (si-NC) siRNA for 24 h, and then the level of Sirt1 protein was measured by western blot. (**h**, **i**) After transfected with pcDNA-HULC or pcDNA3.1 for 18 h, HepG2 cells were treated with 10 μg/ml cycloheximide (CHX) for the indicated times. Then the level of Sirt1 protein was detected by western blot (**h**) and quantified by quantity-one software (**i**). (**j**, **k**) After transfected with si-HULC or si-NC for 18 h, HepG2 cells were treated with 10 μg/ml CHX for the indicated times. Then the level of Sirt1 protein was analyzed and quantified as described in **h** and **i**. (**l**, **m**) After transfected with si-HULC or si-NC for 22 h, HepG2 (**l**) and Hep3B (**m**) cells were treated with or without 20 μM MG132 for 2 h. Then the level of Sirt1 protein was tested by western blot. Tubulin was used as a loading control in all western blot assays. $^{**}P < 0.01$; $^{***}P < 0.001$. ns, no significance; qRT-PCR, quantitative real-time PCR.

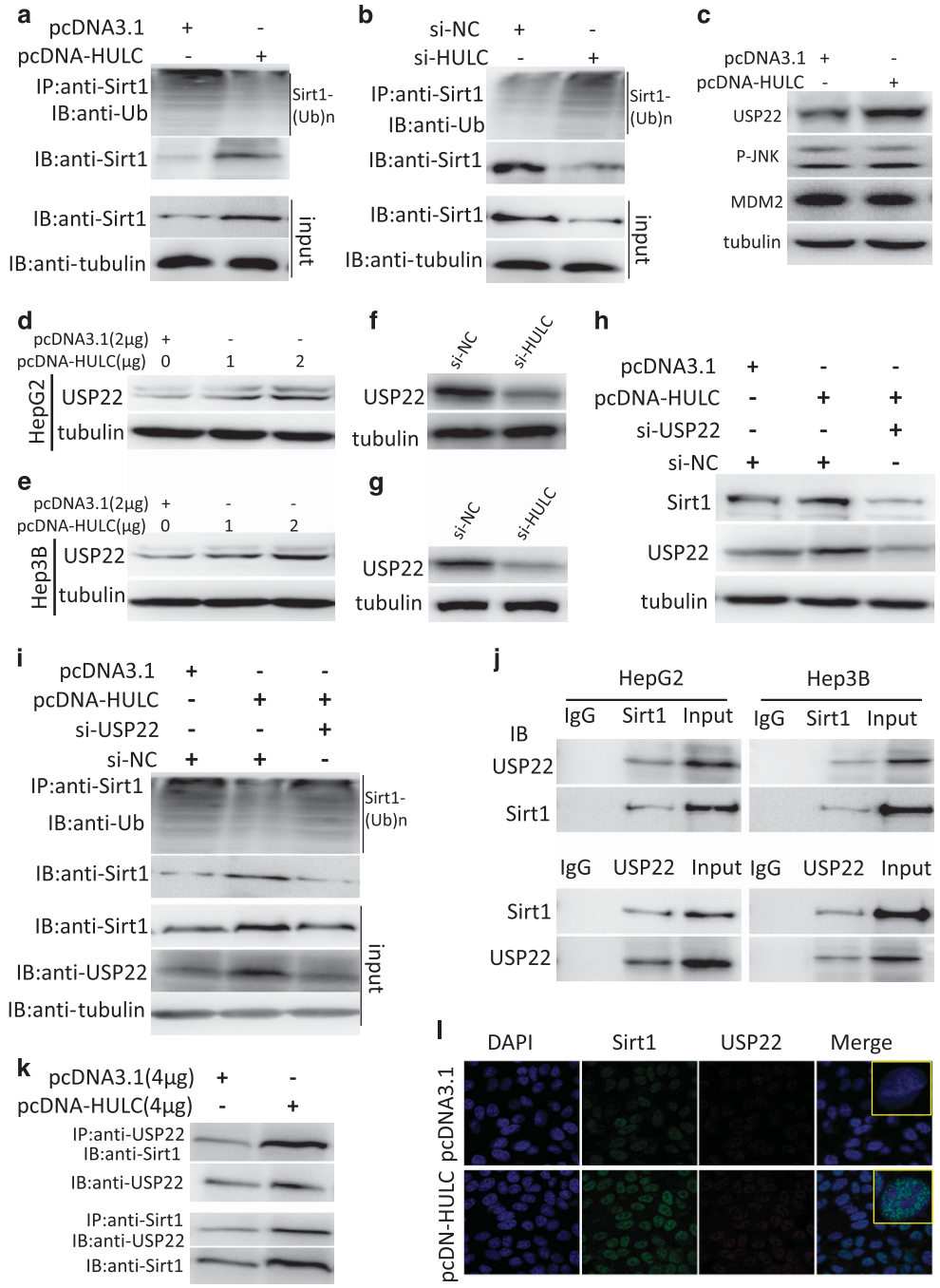


**Figure 3.** HULC promotes Sirt1 deubiquitination through inducing USP22. (**a**) HepG2 cells were transfected with pcDNA-HULC or control vector (pcDNA3.1) for 24 h, and then Sirt1 protein was immunoprecipitated (IP) by rabbit antibody against human Sirt1. Subsequently, the immunoprecipitated Sirt1 (the second panel) was detected with mouse antibody against human Sirt1 by western blot (immunoblotting, IB). Moreover, the ubiquitination of Sirt1 (the first panel) in the immunoprecipitated fraction was measured with IB using mouse antibody against human ubiquitin. Meanwhile, the level of Sirt1 protein in the whole-cell lysates was determined by western blot. (**b**) HepG2 cells were transfected with si-HULC or control (si-NC) siRNA for 24 h. Then all the assays were performed as in **a**. (**c**) HepG2 cells were transfected as in **a**, and then the protein levels of USP22, MDM2 and p-JNK were separately examined by western blot in HepG2 cells. (**d**, **e**) After transfected with pcDNA3.1 or various doses of pcDNA-HULC for 24 h, the level of USP22 protein was detected by western blot in HepG2 (**d**) and Hep3B (**e**) cells. (**f**, **g**) Western blot analysis of USP22 protein after transfected with si-HULC or si-NC for 24 h in HepG2 (**f**) and Hep3B (**g**) cells. (**h**, **i**) HepG2 cells were transfected with pcDNA-HULC or pcDNA3.1 in the presence of si-USP22 or si-NC for 24 h. Then western blot analysis for Sirt1 and USP22 in the whole-cell lysates (**h**), and IP and IB assays for Sirt1 ubiquitination (**i**) as described in **a**. (**j**) Co-immunoprecipitation (Co-IP) assay for the interaction between USP22 and Sirt1 in HepG2 and Hep3B cells. (**k**) Co-IP analysis for the interaction between USP22 and Sirt1 in HepG2 cells after transfected with pcDNA-HULC or pcDNA3.1 for 24 h. (**l**) Double immunofluorescent staining of Sirt1 and USP22 after transfected with pcDNA-HULC or pcDNA3.1 for 24 h in HepG2 cells.

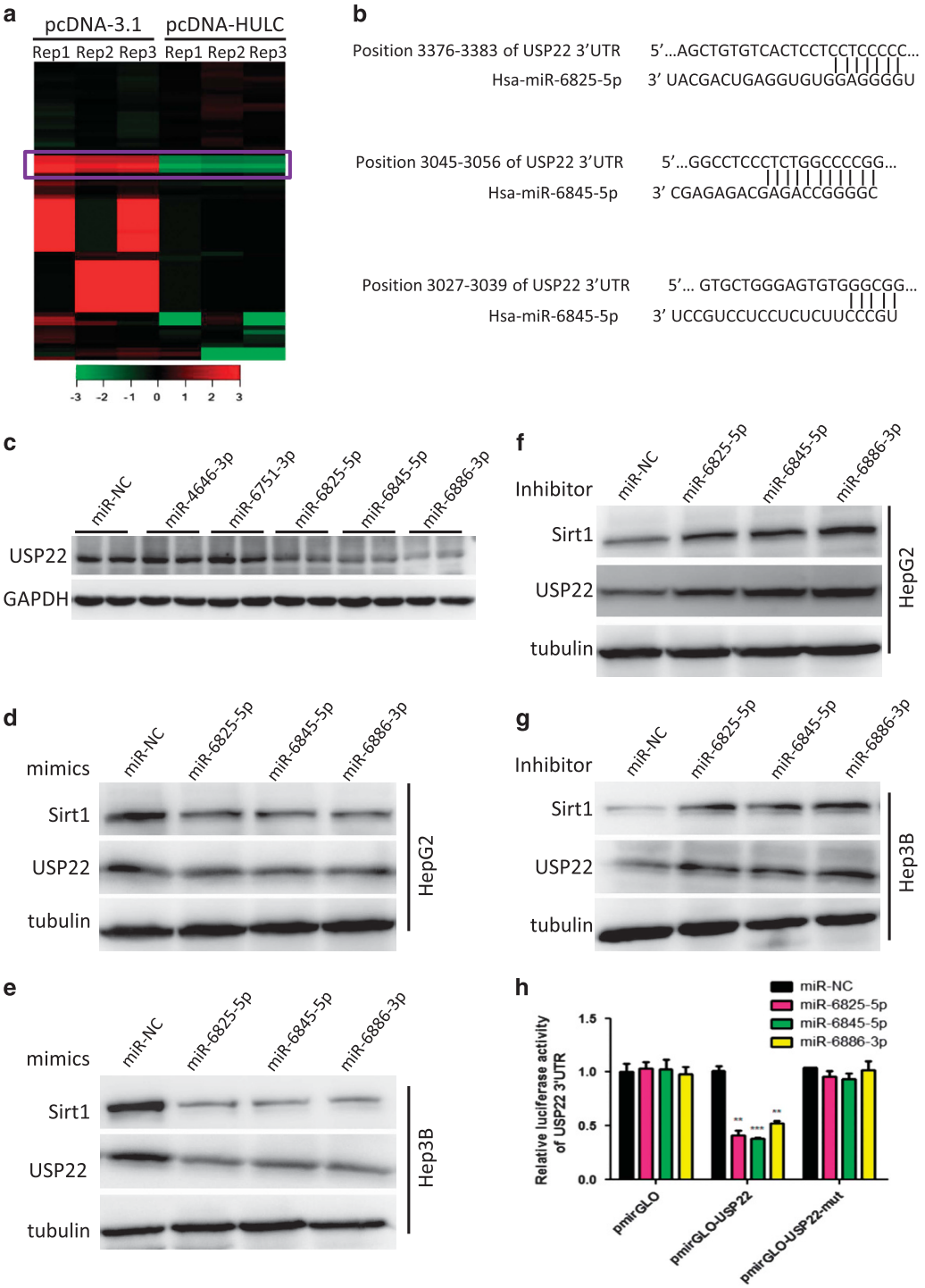


**Figure 4.** miR-6825-5p, miR-6845-5p and miR-6886-3p target and suppress USP22. (**a**) After transfected with pcDNA-HULC or pcDNA3.1 for 24 h in HepG2 cells, the total RNA was extracted and the microarray assay was performed. (**b**) The putative binding sites of miR-6825-5p and miR-6845-5p in 3′-UTR of human USP22 mRNA were predicted with TargetScan. Meanwhile, the potential binding site of miR-6886-3p at USP22 3′-UTR was also shown. (**c**) After separately transfected with the mimics of five candidate miRNAs for 24 h in HepG2 cells, the level of USP22 protein was examined by western blot. (**d**–**g**) HepG2 (**d**, **f**) and Hep3B (**e**, **g**) cells were separately transfected with the mimics (**d**, **e**) or inhibitors (**f**, **g**) of the three miRNAs (miR-6825-5p, miR-6845-5p and miR-6886-3p) for 24 h. Then USP22 and Sirt1 were measured by western blot. (**h**) The mimics of the three miRNAs or control miRNA (miR-NC) were cotransfected with pmirGLO, pmirGLO-USP22 or pmirGLO-USP22-mut in HepG2 cells for 24 h. Then the dual-luciferase reporter assay was performed, and the firely luciferase activity was normalized by renilla luciferase activity. $^{**}P < 0.01$; $^{***}P < 0.001$.

was markedly attenuated by miR-6825-5p, miR-6845-5p and miR-6886-3p, respectively (Figure 5g). These results indicated that HULC can repress the expression and activity of the three miRNAs.

It has been reported that lncRNA can serve as a competing endogenous sponge RNA to interact with miRNAs and decrease the level of miRNA.[10,19] Therefore, RNAhybrid was used to predict the potential binding sites between HULC and the three miRNAs,

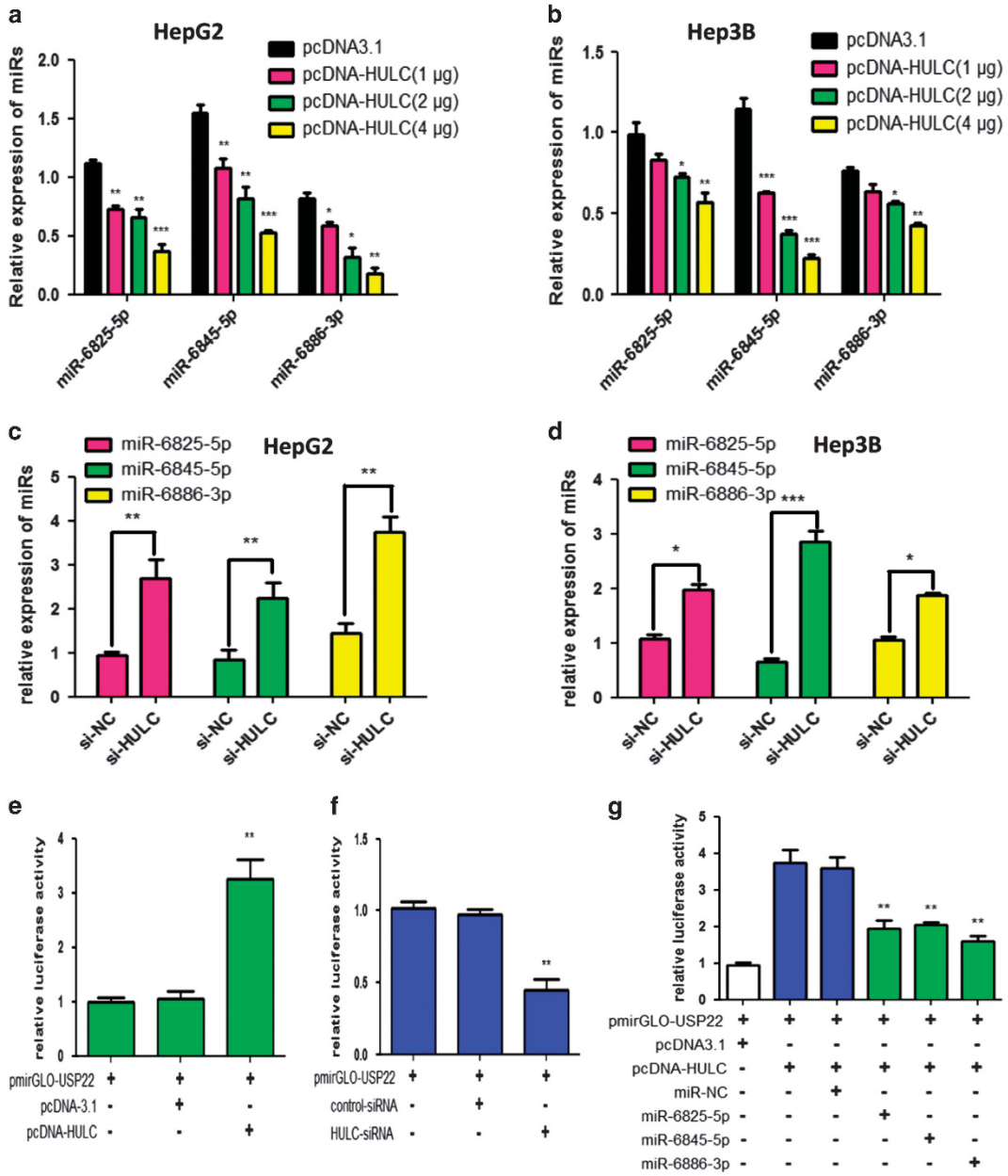


**Figure 5.** HULC inhibits the expression and activity of miR-6825-5p, miR-6845-5p and miR-6886-3p. (**a**, **b**) HepG2 (**a**) and Hep3B (**b**) cells were transfected with pcDNA3.1 or various concentrations of pcDNA-HULC for 24 h. Then the levels of miR-6825-5p, miR-6845-5p and miR-6886-3p were examined by qRT-PCR. (**c**, **d**) qRT-PCR analysis of the three miRNAs after transfected with si-HULC or control (si-NC) siRNA for 24 h in HepG2 (**c**) and Hep3B (**d**) cells. (**e**) After cotransfected with pcDNA-HULC (or pcDNA3.1) and pmirGLO-USP22 for 24 h in HepG2 cells, the dual-luciferase reporter assay was performed, and the firefly luciferase activity was normalized by renilla luciferase activity. (**f**) HepG2 cells were cotransfected with si-HULC or control (si-NC) siRNA and pmirGLO-USP22 for 24 h. Then the luciferase activity was determined as described in **e**. (**g**) HepG2 cells were cotransfected with the mimics of the three miRNAs or control (miR-NC) and pmirGLO-USP22 in the presence of pcDNA-HULC or pcDNA3.1 for 24 h. Then the luciferase activity was detected as described in **e**. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. qRT-PCR, quantitative real-time PCR.

respectively, and the prediction results were shown in Supplementary Figures 4a and b. Subsequently, luciferase reporter containing the full length of HULC was constructed and the luciferase reporter assay was performed. Interestingly, we found that overexpression of the three miRNAs had no influence on the luciferase reporter activity of pmirGLO-HULC (Supplementary Figure 4C), indicating that HULC does not directly interact with any one of the three miRNAs. Namely, HULC does not act as a miRNA sponge to suppress the expression and activity of the three miRNAs. Furthermore, we found that HULC didn't affect the stability of the three miRNAs using actinomycin D (Supplementary Figure 4D), suggesting that HULC may downregulate the three miRNAs through transcription inhibition or epigenetic regulation.

## HULC induces autophagy via upregulating Sirt1 protein

The above studies have demonstrated that HULC stabilizes Sirt1 protein (Figure 2), while previous reports have shown that Sirt1 contributes to autophagy through regulating different autophagy-related proteins and pathways.[20–22] Therefore, we tested whether HULC-mediated upregulation of Sirt1 protein can induce autophagy in HCC cells. As shown in Figures 6a-d, overexpression of

HULC dramatically induced green fluorescent GFP-LC3 punctas (Figures 6a and b), and increased the doube- or muti-membrane compartments (autophagosomes; Figure 6c and d) in HCC cells, which was markedly attenuated by silence of Sirt1 with the siRNA si-Sirt1-1 (Figure 6h-k; si-Sirt1-1 was used to silence Sirt1 in the subsequent experiments). It has been reported that Sirt1 induces

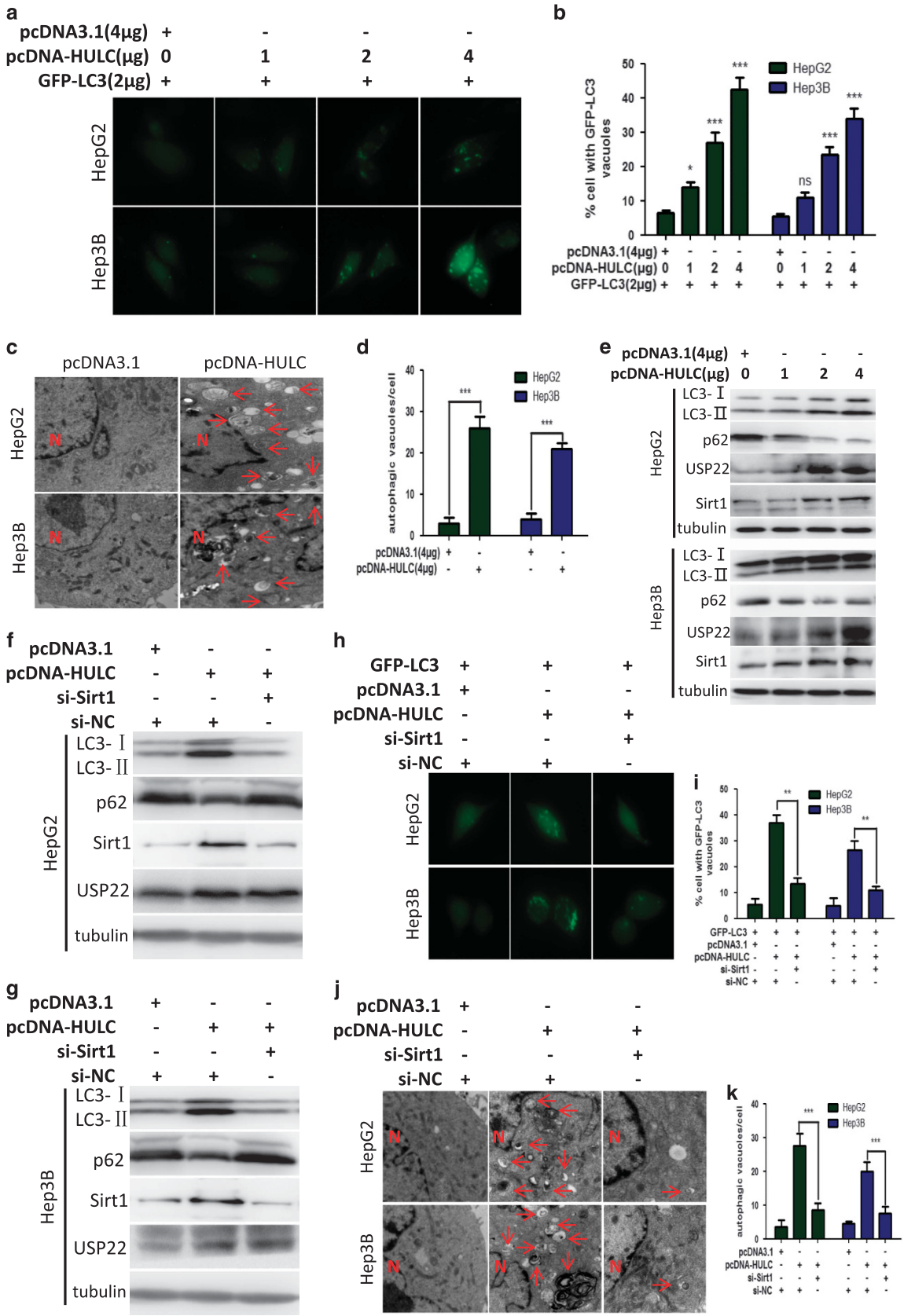


**Figure 6.** For caption see page 3536.

autophagy via suppressing the acetylation of the autophagy-related proteins including Atg5 and Atg7.[20] So we investigated the effect of HULC on the acetylation of Atg5 and Atg7. As shown in Supplementary Figures 6A and B, ectopic expression of HULC significantly decreased the acetylation of Atg5 and Atg7 in HCC cells, and enhanced LC3-II conversion and decreased p62 expression (Figure 6e), which was remarkably alleviated by silence of Sirt1 (Figures 6f and g and Supplementary Figures 6C and D). All of the above data indicated that HULC induces autophagy of HCC cells via upregulating Sirt1 protein.

In addition, overexpression of Sirt1 alone increased the green fluorescent GFP-LC3 puncta (Supplementary Figure 6E), and silence of Sirt1 alone decreased LC3-II level (Supplementary Figure 6F), indicating that Sirt1 has an important role in autophagy induction. Moreover, silencing of USP22 attenuated HULC-mediated upregulation of Sirt1 and LC3-II (Supplementary Figure 6G), indicating that USP22 worked upstream of Sirt1.

### The 'HULC/Sirt1/autophagy' pathway attenuates the chemosensitivity of HCC cells

As shown in Supplementary Figure 5C and D, ectopic expression of HULC promoted the proliferation of HCC cells, which is consistent with the prior study.[12] Moreover, we for the first time characterized that HULC had an pivotal role in the sensitivity of HCC cells in response to chemotherapy. As shown in Figure 7a, antitumor reagents (oxaliplatin, 5-FU and THP) dramatically upregulated the expression of HULC. Oxaliplatin decreased the acetylation of Atg5 and Atg7 (Supplementary Figure 7A) and the level of p62 (Figure 7b), while increased the levels of cleaved-PARP (C-PARP), Sirt1, USP22 and LC3-II (Figure 7b). All the above effects of oxaliplatin were markedly attenuated by silence of HULC (Supplementary Figure 7B and Figure 7c). In addition, silence of HULC also upregulated the level of C-PARP and enhanced the cell growth inhibition in response to 5-FU and THP, respectively (Supplementary Figures 7C-E). Furthermore, knockdown of HULC enhanced oxaliplatin-mediated inhibition of cell survival, which was alleviated by overexpression of HULC, USP22 and Sirt1, respectively (Figures 7d and f). Subsequently, silence of HULC (or USP22 or Sirt1) or inhibition of autophagy enhanced oxaliplatin-induced apoptosis (Figures 7e and g-h). Collectively, these results indicated that the 'HULC-USP22-Sirt1-autophagy' pathway decreases the chemosensitivity of HCC cells.

### HULC alleviates the sensitivity of HCC cells to antitumor reagents *in vivo*

To evaluate the above phenomenon *in vivo*, we established the xenograft tumor models in nude mice using HepG2 cell line with or without stable knockdown of HULC (Supplementary Figures 8A-N) or Sirt1 (The shRNA primers for HULC and Sirt1 were supplied in Supplementary Table 4). Subsequently, we found that silence of HULC (Figures 8a-c) or Sirt1 (Supplementary Figures 9A-D) markedly reduced the xenograft tumor growth, which was further enhanced by the addition of oxaliplatin. Quantitative real-time PCR analysis showed that oxaliplatin increased the expression of HULC in the xenograft tumors, which was markedly attenuated by HULC knockdown (Figure 8d). Immunohistochemistry staining revealed that silence of HULC dramatically alleviated oxaliplatin-mediated upregulation of Sirt1 and USP22 as well as the downregulation of p62, leading to Caspase-3 increase and Ki-67 decrease (Figure 8e). These results indicated that knockdown of HULC or Sirt1 can sensitize HCC to oxaliplatin *in vivo*.

In addition, silencing of Sirt1 dramatically weakened the oxaliplatin-mediated Sirt1 induction and p62 downregulation in the xenograft tumors, leading to Caspase-3 increase and Ki-67 decrease (Supplementary Figure 9E), indicating that Sirt1 plays an important role in oxaliplatin-induced autophagy *in vivo*.

## DISCUSSION

In this study, we have demonstrated that HULC triggers protective autophagy in HCC cells via stabilizing Sirt1 protein, which weakens the sensitivity of HCC cells in response to chemotherapeutic reagents. Specifically, we found that the mechanism by which HULC elevates Sirt1 protein is mediated by the 'three miRNAs (miR-6825-5p, miR-6845-5p and miR-6886-3p)-USP22' pathway (summarized in Supplementary Figure 10). These findings reveals a potential role of HULC in autophagy regulation and chemoresistance of HCC cells.

The protective autophagy has been proved as a potential mechanism for chemoresistance of cancer cells.[23–24] However, it is unclear whether the lncRNA-induced autophagy is involved in the chemosensitivity of HCC cells. Here, we for the first time showed that the HULC-triggered protective autophagy attenuated the sensitivity of HCC cells to chemotherapeutic reagents. In addition, we found that HULC elicited autophagy via upregulating Sirt1 protein (Figure 6). It is well known that Sirt1 induces autophagy through regulating many key autophagy components such as Atg5, Atg7, Atg8, Beclin I, FoxO1, p53 and so on.[25] Our results revealed that HULC promoted the autophagy of HCC cells via enhancing Sirt1-mediated deacetylation of Atg5 and Atg7 (Supplementary Figure 6). Furthermore, previous reports ascribes the high expression of Sirt1 in HCC to its post-translational regulation because Sirt1 mRNA has no significant difference between HCC and adjacent non-cancerous tissues.[15,26] Meanwhile, another study shows that the stability of Sirt1 protein is enhanced in HCC,[27] but the corresponding mechanism is still unknown. In the present study, we for the first time verified that the upregulation of HULC, at least partially, accounted for the elevation of Sirt1 protein in HCC. Subsequently, USP22 was identified to be involved in HULC-mediated Sirt1 induction. Meanwhile, it needs to be noted that MDM2 seemed to be a slightly reduction after HULC overexpression. Recently, it is reported that MDM2 enhances the ubiquitination of Sirt1 and

**Figure 6.** HULC promotes autophagy via upregulating Sirt1 protein. (**a**, **b**) HepG2 and Hep3B cells were cotransfected with pcDNA3.1(or various concentrations of pcDNA-HULC) and GFP-LC3 vector for 24 h. Then the green fluorescent GFP-LC3 puncta (which occurred upon autophagy induction) were observed under a fluorescence microscope. The representative images are shown in **a** and the percentage of the cells with GFP-LC3 puncta was quantified in **b**. (**c**, **d**) After separately transfected with pcDNA-HULC or pcDNA3.1 for 24 h in HepG2 and Hep3B cells, the autophagic vacuoles (autophagosomes) were detected with transmission electron microscopy (TEM). The representative TEM images were shown and the typical autophagosomes were marked with red arrows (**c**). The number of autophagosomes per cell was calculated by counting the number of double-membrane organelles in 10 cells (**d**). (**e**) HepG2 or Hep3B cells were transfected with pcDNA3.1 or various concentrations of pcDNA-HULC for 24 h. Then the protein levels of LC-3, p62, USP22 and Sirt1 were assayed by western blot. (**f**, **g**) HepG2 (**f**) or Hep3B (**g**) cells were cotransfected with si-Sirt1 or si-NC and pcDNA-HULC or pcDNA3.1 for 24 h. Then the protein levels of LC-3, p62, USP22 and Sirt1 were detected by western blot. (**h**, **i**) HepG2 and Hep3B cells were cotransfected as in **f** and **g** in the presence of GFP-LC3 vector for 24 h. Then the green fluorescent GFP-LC3 puncta were photographed and quantified as in **a** and **b**. (**j**, **k**) HepG2 and Hep3B cells were treated as in **f** and **g**, and then the autophagosomes were observed under TEM and quantified as in **c** and **d**. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

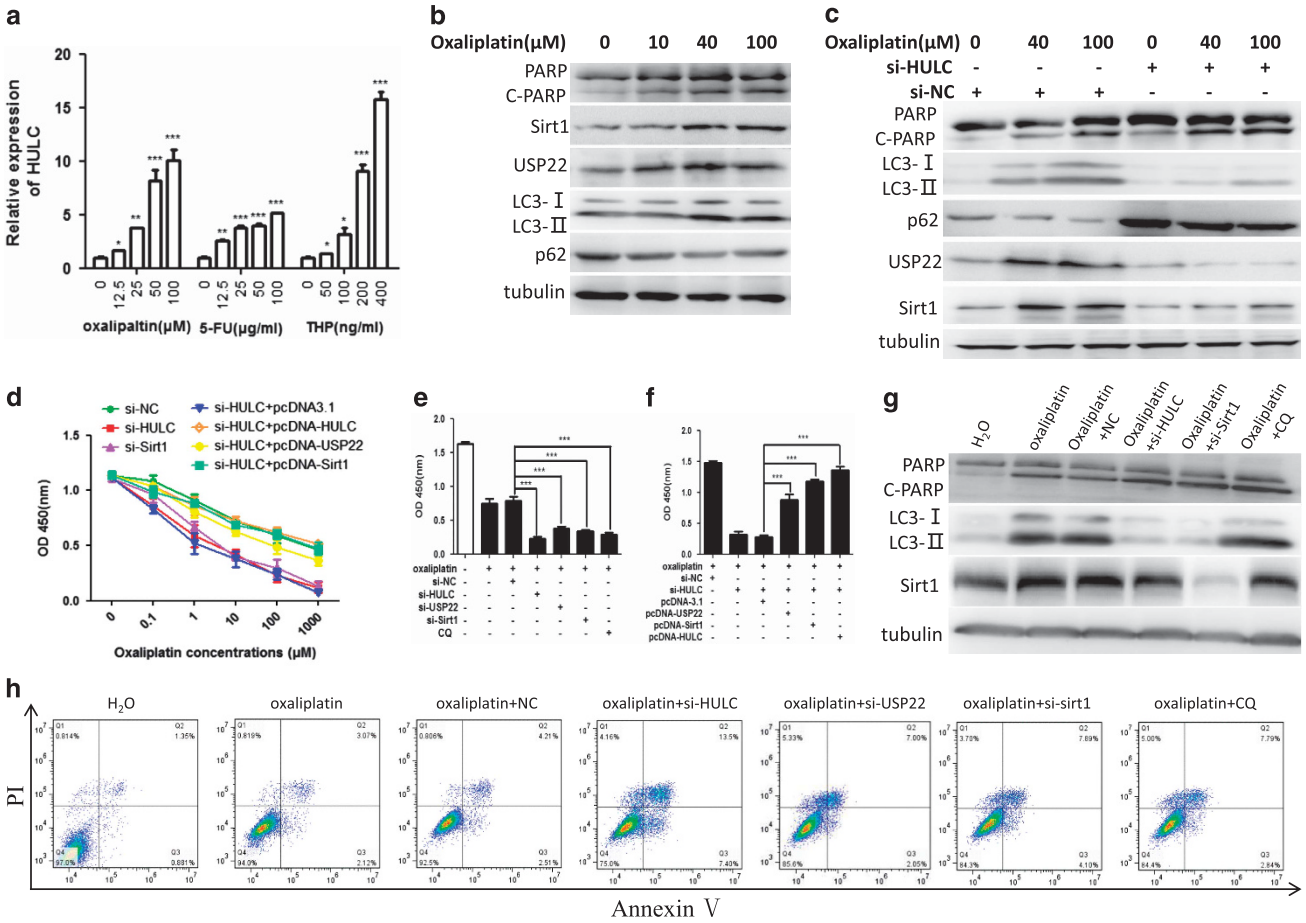


**Figure 7.** The HULC/Sirt1/autophagy pathway attenuates chemosensitivity of HCC cells. (**a**) HepG2 cells were treated with various concentrations of oxaliplatin, 5-FU or THP for 24 h, and then the expression of HULC was examined by qRT-PCR. (**b**) After treatment with the indicated concentrations of oxaliplatin for 24 h, the levels of C-PARP, Sirt1, USP22, LC3 and p62 in HepG2 cells were measured by western blot. (**c**) After transfected with si-HULC or control (si-NC) siRNA for 12 h, HepG2 cells were treated with oxaliplatin for 24 h. Then the levels of C-PARP, Sirt1, USP22, LC3 and p62 were determined by western blot. (**d**) After transfected with si-HULC or si-Sirt1 siRNA in the presence or absence of pcDNA-HULC (or pcDNA-USP22 or pcDNA-Sirt1) for 12 h, HepG2 cells were treated with various concentrations of oxaliplatin for 24 h. Then the cell viability was determined by CCK-8 assay. (**e**) HepG2 cells was transfected with si-HULC, or si-USP22 or si-Sirt1 for 12 h, or pretreatment with autophagy inhibitor CQ (20 μM) for 2 h. Then the cells were treated with oxaliplatin (40 μM) for 24 h, and the cell viability was detected by CCK-8 assay. (**f**) After transfected with si-HULC in the presence or absence of pcDNA-HULC (or pcDNA-USP22 or pcDNA-Sirt1) for 12 h, HepG2 cells were treated with oxaliplatin (40 μM) for 24 h. Then the cell viability was measured by CCK-8 assay. (**g**) HepG2 cells were transfected with si-HULC (or si-Sirt1) siRNA for 12 h, or pretreatment with autophagy inhibitor CQ (20 μM) for 2 h. Then the cells were treated with oxalipaltin (40 μM) for 24 h, and western blot was performed to analyze the protein levels of PARP, LC3 and Sirt1. (**h**) After treated as in **e**, HepG2 cells were stained with annexin V-FITC/PI and analyzed using flow cytometry. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. qRT-PCR, quantitative real-time PCR.

promotes its degradation during DNA damage-induced cell death,[16] which may provide another means by which HULC regulates Sirt1 besides USP22. Of course, it needs further studies to clarify whether MDM2 is involved in the HULC-mediated Sirt1 induction.

USP22, a member of deubiquitinases (DUBs), can catalyze the removal of ubiquitin from target proteins. As part of the transcriptional regulatory histone acetylation and deubiquitination complex known as Spt-Ada-Gcn5 acetyltransferase (SAGA), USP22 participates in the deubiquitination of NAD-dependent protein deacetylase Sirt1,[17] as well as histones H2A and H2B.[28] Recently, mounting evidences have shown that USP22 is involved in oncogenesis associated with poor prognosis in a variety of cancers including HCC.[29,30] Moreover, USP22 has been reported to mediate c-Myc-induced chemoresistance of human FLT3-ITD acute myeloid leukemia stem Cells.[31] However, the regulation of USP22 is still unclear. In the present study, we demonstrated that the lncRNA HULC could dramatically upregulate the protein level of USP22 (Figures 3d and e), and USP22 had a pivotal role in mediating the HULC-induced deubiquitination and stabilization of Sirt1 in HCC cells. However, previous reports have shown that USP22 exerts divergent roles on the regulation of Sirt1 protein.[17,32] The discrepancy among these studies may ascribe to the background of various cells or the different stimulating factors to cells. Moreover, we screened out three novel miRNAs (miR-6825-5p, miR-6845-5p and miR-6886-3p) and proved that they could target USP22 3′-UTR directly (Figures 4a-g). Interestingly, we found that miR-6886-3p inhibited USP22 expression although this miRNA was not predicted to target USP22 3′-UTR. It has been reported that 6- to 8-nt-long fragment at the 5′-end of the miRNA known as 'seed region' is necessary to target mRNA and elicit a response (inhibits mRNA translation or promotes mRNA degradation).[33] However, mounting evidences show that 'noncanonical' sites, which are not perfectly complementary to the miRNA seed region yet are effective in inhibiting gene expression, are exists and account for ~60% of seed interactions.[34,35] So miR-6886-3p inhibited USP22 expression should be in a non-canonical manner in HCC cells.

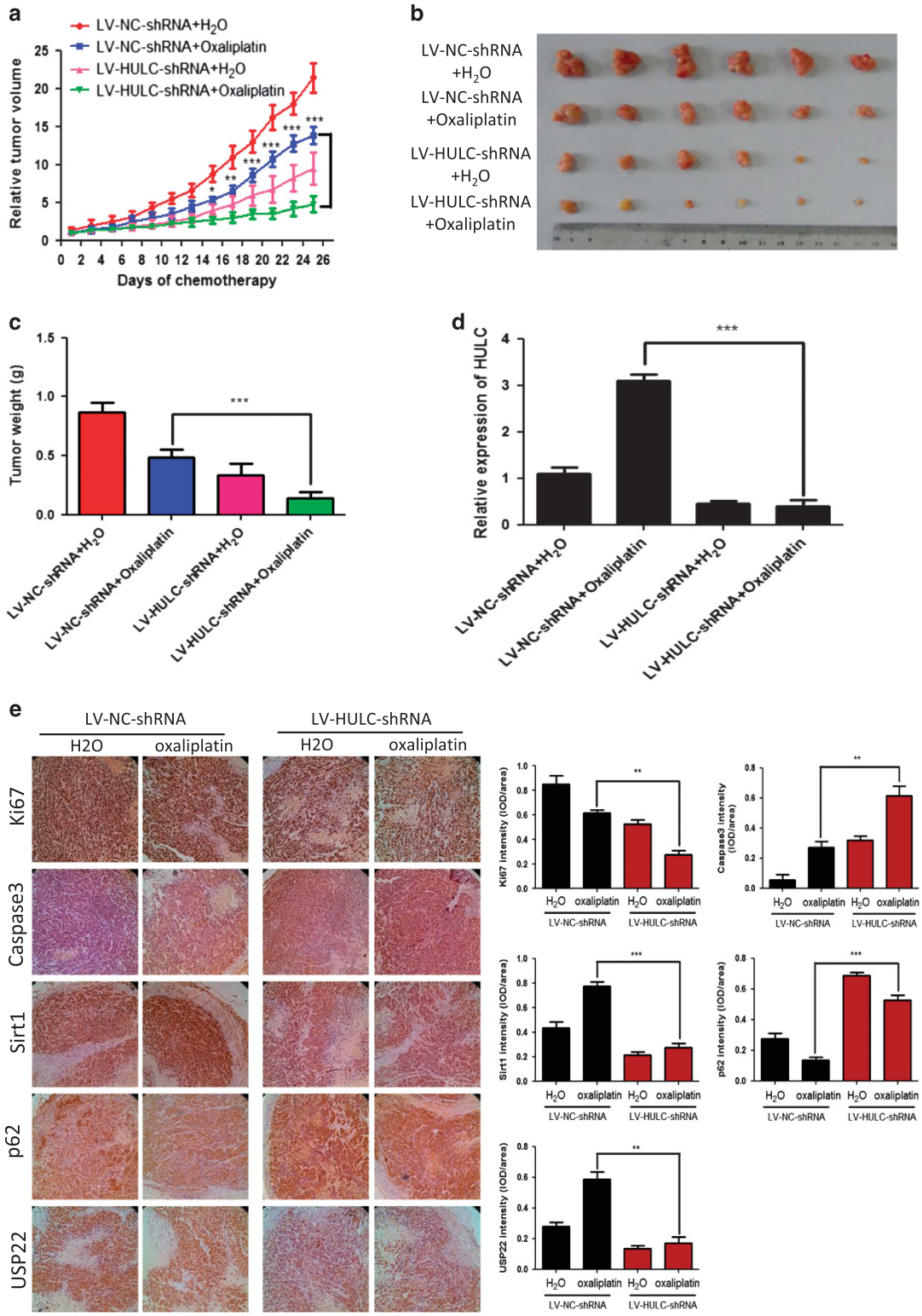


**Figure 8.** Upregulation of HULC weakens the sensitivity of HCC cells to oxaliplatin *in vivo*. (**a**–**e**) $5 \times 10^6$ HepG2 cells with or without HULC stable knockdown were separately injected into the right axilla of nude mice of each group ($n = 12$ per group). When palpable tumors formed, the mice bearing the HepG2 cells with or without HULC stable knockdown were separately randomized into two sub-groups ($n = 6$ per sub-group). Then oxaliplatin (10 mg/kg) or sterile water ($H_2O$) was intraperitoneally injected into the mice of each sub-group twice a week (totally seven times of injection). Subsequently, the xenograft tumor size was monitored every other day (volume = width$^2$ × length × 1/2) (**a**). After 25 days, the xenograft tumors were excised from the nude mice, and then photographed (**b**) and their weights were shown in **c**. The level of HULC in the xenograft tumors was measured by qRT-PCR (**d**). The levels of Ki-67, Casepase3, Sirt1, p62 and USP22 in the xenograft tumors were examined by immunohistochemical staining and quantified with Image ProPlus (IPP) solftware (Media Cybernetics, Rockville, MD, USA) (**e**). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. qRT-PCR, quantitative real-time PCR.

Previous reports have shown that the lncRNA HULC can reduce the expression or activity of miRNAs by acting as competing endogenous sponge RNA.[10,36] Besides the sponge effect, HULC has been reported to elicit methylation of CpG islands in *miR-9* gene promoter, leading to the suppression of miR-9 targeting of PPARA mRNA.[13] In the present study, we found that HULC inhibited the expression and activity of miR-6825-5p, miR-6845-5p and miR-6886-3p not via directly interacting with the three miRNAs (Supplementary Figure 4). We postulate that HULC may suppress the three miRNAs by transcriptional or epigenetic regulation. So it needs further studies to clarify the detailed mechanism (s) by which HULC represses the three miRNAs.

In summary, we revealed in this study that HULC decreases the expression and activities of the three miRNAs (miR-6825-5p, miR-6845-5p and miR-6886-3p), elevates the level of USP22 protein, enhances the deubiquitination of Sirt1 and stabilizes it, which ultimately triggers the autophagy of HCC cells. Furthermore, the HULC/Sirt1/autophagy pathway is activated in the presence of chemotherapeutic reagents, and interference of the pathway enhances the chemosensitivity of HCC cells. Altogether, these findings illustrate a new function of HULC and the 'HULC/USP22/Sirt1/protective autophagy' pathway may serves as a novel target for developing sensitizing strategy to HCC chemotherapy.

## MATERIALS AND METHODS

### Materials

The GFP-LC3 expression vector was kindly provided by Drs N Mizushima and T Yoshimori of Osaka University in Japan. Cycloheximide (CHX) (1014554) was purchased from Xiya Reagent Corporation (Chengdu, China). Chloroquine (CQ) (C6628) and the antibody against LC3 (L7543) were bought from Sigma-Aldrich corporation (St Louis, MO, USA). The antibodies against Sirt1 (#2496) and JNK1/2 (#4668) were from Cell Signaling Technology corporation (Boston, MA, USA). The antibodies against USP22 (ab195289), Sirt1 (ab110304), Atg5 (ab108327), Atg7 (ab52472), p62/SQSTM1 (ab109012) and acetyl lysine (ab22550) were obtained from Abcam corporation (Cambridge, Massachusetts, USA). The antibodies of ki-67 (NB600-1209) and caspase-3 (NB100-56113) were from Novus Biologicals Corporation (Littleton, CO, USA). The antibody of USP22 (AP2148B) was from Abgent company (San Diego, CA, USA). The antibodies against MDM2 (sc965) and ubiquitin (sc-271289) were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The mimics and inhibitors of miR-6825-5p, miR-6845-5p and miR-6886-3p, the negative controls (miR-NC) of mimics and inhibitors were synthesized by GenePharma (Shanghai, China). The sequences of the above RNA oligos were listed in Supplementary Table 1.

### HCC tissues and adjacent non-cancerous specimens

A total of 40 HCC tissues and the corresponding adjacent non-cancerous specimens were obtained from Department of Hepatobiliary Surgery, Xinqiao Hospital, Third Military Medical University (Chongqing, China). Fresh tissue samples were collected and snap frozen in liquid nitrogen. The study was approved by the ethics committee of Third Military Medical University.

### Cell lines and cell culture

Human HCC cell lines including HepG2, Hep3B, PLC, Huh7, smmc7721 and hepatic cell line L02 were originally tested and authenticated by American Type Culture Collection (Manassas, VA, USA) and passaged $<6$ months in the laboratory. All the cells were cultured in DMEM medium containing 10% fetal bovine serum at 37 °C in 5% $CO_2$ incubator.

### Construction of plasmids

The DNA fragment encoding lncRNA HULC was chemically synthesized and inserted into pcDNA-3.1 (Invitrogen, Carlsbad, CA, USA) expression vector after digestion with *Eco*R I and *Xho* I, and the resulting plasmid was named as pcDNA-HULC. The DNA fragments separately encoding wild-type and mutant USP22 3′-UTR were synthesized and cloned into pmir-GLO vector (Thermo Scientific, Waltham, MA, USA) respectively, after digestion with *Pme* I and *Xho* I, and the resulting plasmids were separately named as pmirGLO-USP22 and pmirGLO-USP22-mut. All the DNA fragments were synthesized by Sangon (Shanghai, China). Moreover, the full length HULC-encoding DNA was also inserted into pmir-GLO vector after digestion with *Pme* I and *Xba* I, and the resulting plasmid was named as pmir-HULC.

### Quantitative RT-PCR (qRT-PCR)

Quantitative real-time PCR assays for HULC, Sirt1, USP22, miR-6825-5p, miR-6845-5p and miR-6886-3p were performed using PimeScript RT-PCR kit (Takara, Kyoto, Japan) according to the manufacturer's instructions. The mRNA level of β-actin was used as a control for HULC, Sirt1 and USP22, and U6 RNA was used as a control for the three miRNAs. The primers were listed in Supplementary Table 2. The detailed description of the quantitative real-time PCR assays appears in the Supplementary Materials and Methods.

### Western blot

The proteins were extracted and their concentrations were measured with a BCA protein assay kit (Beyotime, Shanghai, China). Then western blot analysis was performed as previously described.[37] The detailed description appears in the Supplementary Materials and Methods.

### SiRNA assay

The siRNAs for human Sirt1 and control siRNA were synthesized from Genepharma. Human USP22 siRNAs and the control siRNA were purchased from Lab-cell Corporation (Chongqing, China). The transfection of siRNA was performed using the Lipofectamine 2000 transfection reagent (Invitrogen) according to the manufacturer's protocol. HepG2 and Hep3B cells were transfected with related siRNAs for 24 h. All the siRNA sequences are supplied in Supplementary Table 3.

### Dual-luciferase reporter assay

Dual-luciferase reporter assay was performed as previously described.[38] The detailed description appears in the Supplementary Materials and Methods.

### Gene microarray for miRNAs

The microarray was performed by CapitalBio Corporation (Beijing, China) using the Agilent (Santa Clara, CA, USA) human miRNA Array V21.0. The total RNA containing small RNAs were extracted with TRIzol reagent (Invitrogen) from the HepG2 cells transfected with pcDNA-HULC or pcDNA3.1 according to the manufacturer's instructions. The miRNA profile was assayed by Agilent miRNA array with each array containing probes interrogating 2549 human mature miRNAs from miRBase R21.0. Briefly, the total RNA (100 ng) was dephosphorylated and ligated with pCp-Cy3. Then the labeled RNA was purified and hybridized to miRNA arrays. The images were scanned with the Agilent microarray scanner, gridded and analyzed using Agilent feature extraction software version 10.10. To select the differentially expressed genes, we used threshold values of $\geqslant 2$ and $\leqslant -2$-fold change and a Benjamini–Hochberg corrected-*P* vlaue of 0.05. The data was $Log_2$ transformed and median centered by genes using the Adjust Data function of CLUSTER 3.0 software (University of Tokyo, Tokyo, Japan), and then further analyzed with hierarchical clustering using average linkage.

### Transmission electron microscopy (TEM)

TEM was performed as previously described.[39] The detailed description appears in the Supplementary Materials and Methods.

### Confocal microscopy

For a detailed description, see the Supplementary Materials and Methods.

### Co-IP assay

Co-immunoprecipitation was performed as previously described,[40] and the detailed description appears in the Supplementary Materials and Methods.

### Xenograft tumor assay in nude mice

For a detailed description, see the Supplementary Materials and Methods.

### Statistical analysis

The data are expressed as means ± s.d. unless otherwise stated. The Student *t*-test was used for the analysis of independent two groups. One-way analysis of variance with the *post hoc* Tukey test was used for the analysis of difference among three or more groups. The correlation between HULC and Sirt1 protein level was analyzed by the Pearson's test. In all cases, 'ns' represents no significance and $P < 0.05$ was considered statistically significant ('*' represents $P < 0.05$, '**' represents $P < 0.01$, '***' represents $P < 0.001$).

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (81672377, 31470066, 81472291 and 81272690).

## REFERENCES

1 El-Serag HB. Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology* 2012; **142**: 1264–1273 e1261.
2 Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M *et al.* Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; **136**: E359–E386.
3 Carew JS, Nawrocki ST, Cleveland JL. Modulating autophagy for therapeutic benefit. *Autophagy* 2007; **3**: 464–467.
4 Song J, Qu Z, Guo X, Zhao Q, Zhao X, Gao L *et al.* Hypoxia-induced autophagy contributes to the chemoresistance of hepatocellular carcinoma cells. *Autophagy* 2009; **5**: 1131–1144.
5 Klionsky DJ. Autophagy: from phenomenology to molecular understanding in less than a decade. *Nat Rev Mol Cell Biol* 2007; **8**: 931–937.
6 Luo T, Fu J, Xu A, Su B, Ren Y, Li N *et al.* PSMD10/Gankyrin induces autophagy to promote tumor progression through cytoplasmic interaction with ATG7 and nuclear transactivation of ATG7 expression. *Autophagy* 2015; **12**: 1355–1371.
7 Guo XL, Hu F, Zhang SS, Zhao QD, Zong C, Ye F *et al.* Inhibition of p53 increases chemosensitivity to 5-FU in nutrient-deprived hepatocarcinoma cells by suppressing autophagy. *Cancer Lett* 2014; **346**: 278–284.
8 Ulitsky I, Bartel DP. lincRNAs: genomics, evolution, and mechanisms. *Cell* 2013; **154**: 26–46.
9 Batista PJ, Chang HY. Long noncoding RNAs: cellular address codes in development and disease. *Cell* 2013; **152**: 1298–1307.
10 Wang J, Liu X, Wu H, Ni P, Gu Z, Qiao Y *et al.* CREB up-regulates long non-coding RNA, HULC expression through interaction with microRNA-372 in liver cancer. *Nucleic Acids Res* 2010; **38**: 5366–5383.
11 Hammerle M, Gutschner T, Uckelmann H, Ozgur S, Fiskin E, Gross M *et al.* Post-transcriptional destabilization of the liver-specific long noncoding RNA HULC by the IGF2 mRNA-binding protein 1 (IGF2BP1). *Hepatology* 2013; **58**: 1703–1712.
12 Du Y, Kong G, You X, Zhang S, Zhang T, Gao Y *et al.* Elevation of highly up-regulated in liver cancer (HULC) by hepatitis B virus X protein promotes hepatoma cell proliferation via down-regulating p18. *J Biol Chem* 2012; **287**: 26302–26311.
13 Cui M, Xiao Z, Wang Y, Zheng M, Song T, Cai X *et al.* Long noncoding RNA HULC modulates abnormal lipid metabolism in hepatoma cells through an miR-9-mediated RXRA signaling pathway. *Cancer Res* 2015; **75**: 846–857.
14 Lu Z, Xiao Z, Liu F, Cui M, Li W, Yang Z *et al.* Long non-coding RNA HULC promotes tumor angiogenesis in liver cancer by up-regulating sphingosine kinase 1 (SPHK1). *Oncotarget* 2016; **7**: 241–254.
15 Chen J, Zhang B, Wong N, Lo AW, To KF, Chan AW *et al.* Sirtuin 1 is upregulated in a subset of hepatocellular carcinomas where it is essential for telomere maintenance and tumor cell growth. *Cancer Res* 2011; **71**: 4138–4149.
16 Peng L, Yuan Z, Li Y, Ling H, Izumi V, Fang B *et al.* Ubiquitinated sirtuin 1 (SIRT1) function is modulated during DNA damage-induced cell death and survival. *J Biol Chem* 2015; **290**: 8904–8912.
17 Lin Z, Yang H, Kong Q, Li J, Lee SM, Gao B *et al.* USP22 antagonizes p53 transcriptional activation by deubiquitinating Sirt1 to suppress cell apoptosis and is required for mouse embryonic development. *Mol Cell* 2012; **46**: 484–494.
18 Gao Z, Zhang J, Kheterpal I, Kennedy N, Davis RJ, Ye J. Sirtuin 1 (SIRT1) protein degradation in response to persistent c-Jun N-terminal kinase 1 (JNK1) activation contributes to hepatic steatosis in obesity. *J Biol Chem* 2011; **286**: 22227–22234.
19 Wang K, Long B, Zhou LY, Liu F, Zhou QY, Liu CY *et al.* CARL lncRNA inhibits anoxia-induced mitochondrial fission and apoptosis in cardiomyocytes by impairing miR-539-dependent PHB2 downregulation. *Nat Commun* 2014; **5**: 3596.
20 Lee IH, Cao L, Mostoslavsky R, Lombard DB, Liu J, Bruns NE *et al.* A role for the NAD-dependent deacetylase Sirt1 in the regulation of autophagy. *Proc Natl Acad Sci USA* 2008; **105**: 3374–3379.
21 Hariharan N, Maejima Y, Nakae J, Paik J, Depinho RA, Sadoshima J. Deacetylation of FoxO by Sirt1 plays an essential role in mediating starvation-induced autophagy in cardiac myocytes. *Circ Res* 2010; **107**: 1470–1482.
22 Song YM, Lee YH, Kim JW, Ham DS, Kang ES, Cha BS *et al.* Metformin alleviates hepatosteatosis by restoring SIRT1-mediated autophagy induction via an AMP-activated protein kinase-independent pathway. *Autophagy* 2015; **11**: 46–59.
23 Shteingauz A, Boyango I, Naroditsky I, Hammond E, Gruber M, Doweck I *et al.* Heparanase enhances tumor growth and chemoresistance by promoting autophagy. *Cancer Res* 2015; **75**: 3946–3957.
24 He J, Yu JJ, Xu Q, Wang L, Zheng JZ, Liu LZ *et al.* Downregulation of ATG14 by EGR1-MIR152 sensitizes ovarian cancer cells to cisplatin-induced apoptosis by inhibiting cyto-protective autophagy. *Autophagy* 2015; **11**: 373–384.
25 Qiu G, Li X, Che X, Wei C, He S, Lu J *et al.* SIRT1 is a regulator of autophagy: Implications in gastric cancer progression and treatment. *FEBS Lett* 2015; **589**: 2034–2042.
26 Bae HJ, Noh JH, Kim JK, Eun JW, Jung KH, Kim MG *et al.* MicroRNA-29c functions as a tumor suppressor by direct targeting oncogenic SIRT1 in hepatocellular carcinoma. *Oncogene* 2014; **33**: 2557–2567.
27 Chen HC, Jeng YM, Yuan RH, Hsu HC, Chen YL. SIRT1 promotes tumorigenesis and resistance to chemotherapy in hepatocellular carcinoma and its expression predicts poor prognosis. *Ann Surg Oncol* 2012; **19**: 2011–2019.
28 Zhang XY, Varthi M, Sykes SM, Phillips C, Warzecha C, Zhu W *et al.* The putative cancer stem cell marker USP22 is a subunit of the human SAGA complex required for activated transcription and cell-cycle progression. *Mol Cell* 2008; **29**: 102–111.
29 Schrecengost RS, Dean JL, Goodwin JF, Schiewer MJ, Urban MW, Stanek TJ *et al.* USP22 regulates oncogenic signaling pathways to drive lethal cancer progression. *Cancer Res* 2014; **74**: 272–286.
30 Tang B, Tang F, Li B, Yuan S, Xu Q, Tomlinson S *et al.* High USP22 expression indicates poor prognosis in hepatocellular carcinoma. *Oncotarget* 2015; **6**: 12654–12667.
31 Li L, Osdal T, Ho Y, Chun S, McDonald T, Agarwal P *et al.* SIRT1 activation by a c-MYC oncogenic network promotes the maintenance and drug resistance of human FLT3-ITD acute myeloid leukemia stem cells. *Cell Stem Cell* 2014; **15**: 431–446.
32 Armour SM, Bennett EJ, Braun CR, Zhang XY, McMahon SB, Gygi SP *et al.* A high-confidence interaction map identifies SIRT1 as a mediator of acetylation of USP22 and the SAGA coactivator complex. *Mol Cell Biol* 2013; **33**: 1487–1502.
33 Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell* 2009; **136**: 215–233.
34 Khorshid M, Hausser J, Zavolan M, van Nimwegen E. A biophysical miRNA-mRNA interaction model infers canonical and noncanonical targets. *Nat Methods* 2013; **10**: 253–255.
35 Helwak A, Kudla G, Dudnakova T, Tollervey D. Mapping the human miRNA interactome by CLASH reveals frequent noncanonical binding. *Cell* 2013; **153**: 654–665.
36 Li SP, Xu HX, Yu Y, He JD, Wang Z, Xu YJ *et al.* LncRNA HULC enhances epithelial-mesenchymal transition to promote tumorigenesis and metastasis of hepatocellular carcinoma via the miR-200a-3p/ZEB1 signaling pathway. *Oncotarget* 2016; **7**: 42431–42446.
37 Hu C, Liu D, Zhang Y, Lou G, Huang G, Chen B *et al.* LXRalpha-mediated down-regulation of FOXM1 suppresses the proliferation of hepatocellular carcinoma cells. *Oncogene* 2014; **33**: 2888–2897.
38 Zhou P, Huang G, Zhao Y, Zhong D, Xu Z, Zeng Y *et al.* MicroRNA-363-mediated downregulation of S1PR1 suppresses the proliferation of hepatocellular carcinoma cells. *Cell Signal* 2014; **26**: 1347–1354.
39 Li Q, Fang Y, Zhu P, Ren CY, Chen H, Gu J *et al.* Burkholderia pseudomallei survival in lung epithelial cells benefits from miRNA-mediated suppression of ATG10. *Autophagy* 2015; **11**: 1293–1307.
40 Hu C, Ni Z, Li BS, Yong X, Yang X, Zhang JW *et al.* hTERT promotes the invasion of gastric cancer cells by enhancing FOXO3a ubiquitination and subsequent ITGB1 upregulation. *Gut* 2015; **66**: 31–42.

Supplementary Information accompanies this paper on the Oncogene website (http://www.nature.com/onc)