Long Noncoding RNA IncARSR Promotes Doxorubicin Resistance in Hepatocellular Carcinoma via Modulating PTEN-PI3K/Akt Pathway

Yaling Li, Yun Ye, Bimin Feng, and Yan Qi

1Department of Pharmacy, The Affiliated Hospital of Southwest Medical University, Luzhou, Sichuan, China
2Central Laboratory, Yunnan Provincial Hospital of Traditional Chinese Medicine, Kunming, Yunnan, China

ABSTRACT

Hepatocellular carcinoma (HCC) is generally resistant to chemotherapy due to intrinsic or acquired drug resistances. Many molecules and signaling pathways are involved in chemo-resistance of HCC cells. However, the contribution of long noncoding RNA (lncRNA) to chemo-resistance of HCC cells is still largely unknown. In this study, we revealed the critical roles of long noncoding RNA IncARSR in chemo-resistance of HCC cells. IncARSR is upregulated in HCC, associated with large tumor size and advanced BCLC stage, and indicates poor prognosis. Functional assays showed that overexpression of IncARSR enhances doxorubicin resistance of HCC cells in vitro and in vivo. And while knockdown of IncARSR increases sensitivity of HCC cells to doxorubicin in vitro and in vivo. Mechanistically, we found that IncARSR physically associates with PTEN mRNA, promotes PTEN mRNA degradation, decreases PTEN expression, and activates PI3K/Akt pathway. PTEN is downregulated in HCC, and the expression of PTEN is negatively correlated with IncARSR in HCC tissues. Furthermore, the effects of IncARSR overexpression on doxorubicin resistance could be reversed by PI3K/Akt pathway inhibitor, and IncARSR knockdown-induced doxorubicin sensitivity could be reversed by PTEN depletion. Taken together, our results showed that upregulated IncARSR promotes doxorubicin resistance in HCC via modulating PTEN-PI3K/Akt pathway, and implied that IncARSR may serve as a promising prognostic biomarker and therapeutic target for HCC chemo-resistance. J. Cell. Biochem. 9999: 1–10, 2017. © 2017 Wiley Periodicals, Inc.

KEY WORDS: LONG NONCODING RNA; lncARSR; HEPATOCELLULAR CARCINOMA; DOXORUBICIN CHEMORESISTANCE; PTEN-PI3 K/Akt PATHWAY

Hepatocellular carcinoma (HCC) is the fifth most common malignancy and the second leading cause of cancer-related death worldwide [Torre et al., 2015]. Surgical resection is the main therapeutic strategy for HCC [Yang and Roberts, 2010]. But only HCC patients at early stage are suitable for surgery. For those advanced HCC, transarterial chemoembolization (TACE) is currently the only proper treatment [Forner et al., 2014]. Doxorubicin is the first-line chemotherapy agent for TACE currently [Peck-Radosavljevic, 2014]. However, the intrinsic or acquired resistances to doxorubicin of HCC cells attenuate the effects of TACE [Zhao et al., 2011]. Therefore, it is necessary to further uncover the underlying molecular mechanisms of doxorubicin resistance, identify molecular biomarkers for predicting doxorubicin sensitivity, and develop suitable therapeutic target for HCC doxorubicin resistance.

With great advancements of genomics and transcriptomics technologies, tens of thousands of previously uncharacterized long noncoding RNAs (lncRNAs) have been identified [Yan et al., 2015]. With more than 200 nucleotides in length, lncRNAs have limited protein coding potential [Novak Kujundzic et al., 2008; Ponting et al., 2009; Huarte, 2015]. However, accumulating evidences reveal that lncRNAs have critical functions in various pathophysiological processes and their dysregulation contributes to many disease states, particular for cancers [Dholpuria et al., 2017; Lin et al., 2016; Liu et al., 2016; Schmitt and Chang, 2016; Zhang et al., 2016; Chen et al., 2017]. In HCC, several lncRNAs have been reported to play oncogenic or tumor suppressive roles through diverse mechanisms. For example, upregulated lncRNA-ATB promotes HCC metastasis via binding miR-200s and IL11 mRNA [Yuan et al., 2014]. Low expressed H19 suppresses metastasis via epigenetically activating miR-200s [Zhang et al., 2013]. Increased GPC3-AS1 promotes HCC progression via activating GPC3 transcription [Zhu et al., 2016]. However, the contribution of lncRNAs to doxorubicin resistance of HCC cells is still unknown.

IncARSR is a recently identified IncRNA, which is located on chromosome 9q21 and has 591 nucleotides in length [Qu et al., 2016a]. IncARSR is reported to be transcriptionally activated by...
AKT, and to promote sunitinib resistance in renal cancer via directly binding miR-34 and miR-449, and thereby upregulating AXL and c-MET [Qu et al., 2016a]. IncARS also promotes renal tumor-initiating cells expansion via physically interacting with YAP and facilitating YAP nuclear translocation [Qu et al., 2016b]. Although IncARS plays important roles in renal cancer, whether IncARS also has functions in other cancers and whether the role of IncARS is renal cancer specific are still largely unknown. Moreover, the expression, clinical significances, and contribution of IncARS to HCC doxorubicin resistance are also unclear.

In the present study, we measured the expression of IncARS in HCC tissues, analyzed its association with clinicopathological features and prognosis. Using in vitro and in vivo gain-of-function and loss-of-function experiments, we investigated the roles of IncARS on doxorubicin resistance of HCC cells. Furthermore, we explored the underlying molecular mechanisms responsible for the effects of IncARS on doxorubicin resistance of HCC cells.

MATERIALS AND METHODS

TISSUE SPECIMENS

A total of 92 pairs of HCC tissues and adjacent noncancerous liver tissues were collected with written informed consent from patients who underwent radical resection at The Affiliated Hospital of Southwest Medical University (Luzhou, China). All patients received no therapy before surgery. All the tissue specimens were diagnosed by two pathologists. Ethical approval was granted from the Ethic Committee of The Affiliated Hospital of Southwest Medical University.

CELL LINES AND REAGENTS

The human HCC cell lines SMMC-7721 and HepG2 were purchased from Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China). These cells were maintained in DMEM supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA) and cultured at 37°C in an atmosphere with 5% CO2. Where indicated, cells were treated with doxorubicin (Selleck, Houston, TX), α-amanitin (Sigma–Aldrich, Saint Louis, MO), or LY294002 (Selleck) at indicated concentration for the indicated time.

RNA ISOLATION AND REAL-TIME QUANTITATIVE POLYMERASE CHAIN REACTION (qPCR)

Total RNA was isolated from tissues or cultured cells using TRIzol Reagent (Invitrogen) according to the manufacturer’s instruction. After being treated with DNase I to remove genomic DNA, the RNA was reverse transcribed into first-strand cDNA using the M-MLV Reverse Transcriptase (Invitrogen). Real-time qPCR was carried out using SYBR Green (Takara, Dalian, China) with the StepOnePlus Real-time PCR system (Applied Biosystems, Foster City, CA). The expression of indicated RNAs was normalized against GAPDH.

IN VITRO CHEMOSENSITIVITY ASSAY

Indicated HCC cells were plated at 3000 cells per well in 96-well plates. After incubation for 48 h, the medium was replaced with medium containing indicated concentration of doxorubicin and cultured for another 24 h. Then cell viability was detected using the Cell Counting Kit–8 (CCK-8) assays (Dojindo Laboratories, Kumamoto, Japan) following the manufacturer’s instructions. The absorbance at 450 nm was measured at 2 h to indicate cell viability.

CELL APOPTOSIS ASSAY

Cell apoptosis was detected using the TdT-mediated dUTP nick end labeling (TUNEL) assays with the Dead End™ Fluorometric TUNEL System (Promega, Madison, WI) following the manufacturer’s protocol. Cells with nuclear green fluorescence labeling were defined as TUNEL-positive cells.
IN VIVO CHEMOSENSITIVITY ASSAY
Indicated HCC cells (3.0 × 10^6) were injected subcutaneously into the flanks of male athymic BALB/c nude mice at 5 weeks of age. From the 14th day after injecting, the mice were intraperitoneally administrated with doxorubicin every 3 days. Tumor growth was detected by measuring the tumor length (L) and width (W), and tumor volume was calculated according to the equation \( V = LW^2/2 \). The animal studies were approved by the Ethic Committee of The Affiliated Hospital of Southwest Medical University.

IMMUNOHISTOCHEMISTRY
Subcutaneously xenografts were immunostained for Ki67. For immunohistochemistry, paraffin embedded sections were deparaffinized, rehydrated, followed by antigen retrieval. After being incubated with Ki67 specific antibody (Abcam, Hong Kong, China) and secondary antibody, the slides were finally visualized with 3, 3-diaminobenzidine.

CYTOPLASMIC AND NUCLEAR RNA ISOLATION
Cytoplasmic and nuclear RNA were isolated and purified with the Cytoplasmic & Nuclear RNA Purification Kit (Norgen, Belmont, CA) in accordance with the manufacturer’s instruction.

RNA PULL-DOWN
IncARSR was in vitro transcribed from pSPT19-IncARSR using the SP6 RNA polymerase (Roche, Mannheim, Germany) and biotin-labeled using Biotin RNA Labeling Mix (Roche). After being treated with RNase-free DNase I (Roche), the in vitro transcribed RNA was purified with an RNeasy Mini Kit (Qiagen, Valencia, CA). Then 1 mg of whole-cell lysates from SMMC-7721 cells were incubated with 3 μg of purified biotinylated IncARSR for 1 h at 25°C, followed by being isolated with streptavidin agarose beads (Invitrogen). The mRNAs present in the pull-down material were quantified by qPCR.

WESTERN BLOT
Proteins were acquired from indicated HCC cells using RIPA Lysis Buffer and PMSF (Beyotime Co., Jiangsu, China) according to the manufacturer’s instructions. Then the proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, followed by being transferred to a PVDF membrane. After being blocked using bovine serum albumin, the blots were incubated with PTEN (Abcam), p-Akt (Cell Signaling Technology, Boston, MA), Akt (Cell Signaling Technology) or GAPDH (Abcam) primary antibodies. After being washed, the blots were further incubated with IRdye 700-conjugated goat anti-mouse IgG or IRdye 800-conjugated goat anti-rabbit IgG, and were detected using an Odyssey infrared scanner (Li-Cor, Lincoln, NE). The expression of proteins was normalized against GAPDH.

STATISTICAL ANALYSIS
All statistical analyses were carried out using the GraphPad Prism Software. For comparisons, Wilcoxon signed-rank test, Log-rank test, Pearson chi-square test, Student’s t-test, Mann–Whitney test, and Pearson correlation analysis were performed as indicated. A P value <0.05 was considered as statistically significant.
From the 14th day after subcutaneous injecting of lncARSR stably depleted or control SMMC-7721 cells, the mice were administrated with doxorubicin (1.5 mg/kg body weight) every 3 days. As shown in Figure 3G, knockdown of lncARSR enhanced the inhibiting effects of doxorubicin on xenograft growth in vivo. Immunohistochemistry staining of Ki67 showed that knockdown of lncARSR further decreased the number of Ki67-positive cells which was reduced by doxorubicin treatment (Fig. 3H). Collectively, these data demonstrated that knockdown of lncARSR increases doxorubicin sensitivity of HCC cells both in vitro and in vivo.

**lncARSR DIRECTLY BINDS TO PTEN mRNA AND DECREASES PTEN EXPRESSION**

Recently, several lncRNAs have been reported to directly bind to target mRNAs and regulate their stability or translation [Faghihi et al., 2008; Stazic et al., 2011; Carrieri et al., 2012]. PTEN is a well-known tumor suppressor and has been reported to be involved in doxorubicin resistance of several cancers [Zhang et al., 2014; Yuan et al., 2015; Zhong et al., 2015]. Intriguingly, we identified three regions of high complementary between lncARSR and PTEN mRNA using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Fig. 4A). To investigate whether lncARSR binds to PTEN mRNA, we first detected the cellular compartmentalization of lncARSR and PTEN mRNA. The results showed that both lncARSR and PTEN mainly localize in cytoplasm (Fig. 4B). Then, we performed RNA pull-down assays using in vitro transcribed biotin-labeled lncARSR. As shown in Figure 4C, lncARSR specifically interacted with PTEN mRNA, but not GAPDH mRNA which does not have complementary regions to lncARSR. To investigate the effects of lncARSR on PTEN mRNA stability via the direct binding, we treated lncARSR stably overexpressed or depleted SMMC-7721 cells with a-amanitin to block novel RNA production and then detected the loss of PTEN and GAPDH mRNA. As shown in Figure 4D, overexpression of lncARSR shortened the half-life of PTEN mRNA and promoted PTEN mRNA degradation. Knockdown of lncARSR elongated the half-life of PTEN mRNA and inhibited PTEN mRNA degradation (Fig. 4E). Furthermore, PTEN mRNA expression is downregulated in lncARSR overexpressed SMMC-7721 cells, and upregulated in lncARSR depleted SMMC-7721 cells (Fig. 4F, G). These data suggested that lncARSR directly binds to PTEN mRNA, promotes PTEN mRNA degradation, and down-regulates PTEN mRNA level.

**TABLE I. Associations Between lncARSR Expression and Clinicopathological Features**

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Low</th>
<th>High</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, year</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤50</td>
<td>44</td>
<td>20</td>
<td>24</td>
<td>0.404</td>
</tr>
<tr>
<td>&gt;50</td>
<td>48</td>
<td>26</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>82</td>
<td>43</td>
<td>39</td>
<td>0.180</td>
</tr>
<tr>
<td>Female</td>
<td>10</td>
<td>3</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>AFP, ng/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>28</td>
<td>18</td>
<td>10</td>
<td>0.070</td>
</tr>
<tr>
<td>&gt;20</td>
<td>64</td>
<td>28</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>HBsAg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>74</td>
<td>37</td>
<td>37</td>
<td>1.000</td>
</tr>
<tr>
<td>Negative</td>
<td>18</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Liver cirrhosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With</td>
<td>60</td>
<td>25</td>
<td>35</td>
<td>0.029*</td>
</tr>
<tr>
<td>Without</td>
<td>32</td>
<td>21</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Tumor size, cm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5</td>
<td>53</td>
<td>32</td>
<td>21</td>
<td>0.020*</td>
</tr>
<tr>
<td>≥5</td>
<td>39</td>
<td>14</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Tumor number</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>64</td>
<td>35</td>
<td>29</td>
<td>0.174</td>
</tr>
<tr>
<td>≥1</td>
<td>28</td>
<td>11</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Microvascular invasion</td>
<td></td>
<td></td>
<td></td>
<td>0.059</td>
</tr>
<tr>
<td>Present</td>
<td>41</td>
<td>16</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>51</td>
<td>30</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>BCLC stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-A</td>
<td>42</td>
<td>26</td>
<td>16</td>
<td>0.036*</td>
</tr>
<tr>
<td>B-C</td>
<td>50</td>
<td>20</td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>

Median expression level of lncARSR was used as the cutoff. P-value was acquired by Pearson chi-square test.

AFP, alpha-fetoprotein; HBsAg, hepatitis B surface antigen; BCLC, Barcelona Clinic Liver Cancer.

*P < 0.05.
PTEN mRNA level in HCC tissues. We first measured PTEN mRNA level in the same 92 pairs of HCC tissues and adjacent noncancerous liver tissues as shown in Figure 1A. PTEN mRNA levels were significantly downregulated in HCC tissues compared with adjacent noncancerous tissues (Fig. 5A). Pearson’s correlation analysis showed that lncARSR expression was negatively correlated with PTEN expression in these HCC tissues (Fig. 5B), supporting the negative regulation of PTEN by lncARSR.

**lncARSR ACTIVATES PI3K/Akt PATHWAY**

PTEN is known to negatively regulate PI3K/Akt pathway and further inhibit tumor progression [Liao et al., 2017; Miao et al., 2017]. Therefore, we further investigated the effects of lncARSR on PTEN-PI3K/Akt pathway. PTEN protein level and Akt phosphorylation level of lncARSR stably overexpressed or depleted SMMC-7721 cells were measured. As shown in Figure 6A and B, overexpression of lncARSR decreased PTEN protein level and increased Akt phosphorylation level, and whereas knockdown of lncARSR increased PTEN protein level and decreased Akt phosphorylation level. Neither overexpression nor depletion of lncARSR changed total Akt protein level. These data demonstrated that lncARSR activates PTEN-PI3K/Akt pathway.

THE EFFECTS OF lncARSR ON DOXORUBICIN RESISTANCE ARE DEPENDENT ON PTEN-PI3K/Akt PATHWAY

To further investigate whether PTEN-PI3K/Akt pathway was involved in lncARSR-induced doxorubicin resistance of HCC cells, we treated lncARSR overexpressed SMMC-7721 cells with PI3K/Akt pathway inhibitor LY294002. CCK-8 assays showed that treatment with LY294002 reversed the resistance of HCC cells to doxorubicin caused by lncARSR overexpression (Fig. 7A). TUNEL staining showed that treatment with LY294002 reversed the decreased doxorubicin-induced apoptosis caused by lncARSR overexpression (Fig. 7B). Furthermore, we stably depleted PTEN in lncARSR knocked-down SMMC-7721 cells (Fig. 7C). CCK-8 assays showed that depletion of PTEN reversed the sensitivity of HCC cells to doxorubicin caused by lncARSR knockdown (Fig. 7D). TUNEL staining showed that depletion of PTEN rescued the increased doxorubicin-induced apoptosis caused by lncARSR knockdown (Fig. 7E). These data suggested that the effects of lncARSR on
doxorubicin responses of HCC cells are dependent on PTEN-P13K/Akt pathway.

**DISCUSSION**

Although chemotherapy is commonly used and shows consistent survival benefit for many cancers, such as lung cancer, breast cancer, osteosarcoma, melanoma, etc., most HCC are not sensitive to chemotherapy [Tacar et al., 2013; Choi et al., 2016; Xi et al., 2016]. Improving the sensitivity of HCC cells to chemotherapy would have great survival benefit for HCC patients. The mechanisms of chemo-resistance are complex and many molecules and signaling pathways are involved in chemo-resistance of HCC cells. ARK5 is reported to promote doxorubicin resistance of HCC cells via inducing epithelial-mesenchymal transition. Aurora-A promotes chemo-resistance of HCC cells via modulating NF-kB/miR-21/PTEN signaling pathway [Xu et al., 2016]. miR-26 enhances chemo-sensitivity of HCC cells via inhibiting autophagy [Jin et al., 2017]. Variant 1 of KIAA0101 promotes chemo-resistance of HCC cells via inhibiting p53 [Liu et al., 2012]. CD166 represses doxorubicin-induced apoptosis via modulating PI3K/Akt and YAP in liver cancer [Ma et al., 2014].

Recently, increasing evidences showed that over 68% of genes are transcribed as lncRNAs [Iyer et al., 2015]. Except the huge amount, lncRNAs are also demonstrated to have important roles [Mercer et al., 2009; Gibb et al., 2011]. In the study of chemo-resistance of HCC cells, people mainly focused their attentions on protein-coding genes and miRNAs. Due to the huge amount and important roles of lncRNAs in HCC, we apparently could not exclude lncRNAs in chemo-resistance of HCC cells. In this study, we revealed that...
Fig. 4. IncARSR interacts with PTEN mRNA and promotes PTEN mRNA degradation. (A) Schematic outlining the regions of putative interaction between IncARSR (subject) and PTEN mRNA (query). (B) IncARSR and PTEN mRNA in subcellular fractions of SMMC-7721 cells were quantified by qPCR. U6 and β-actin were used as nucleus and cytoplasm marker, respectively. (C) SMMC-7721 cell lysates were incubated with biotin-labeled IncARSR; after pull-down, mRNA was extracted and detected by qPCR. Data are shown as a percentage of input RNA. (D, E) The stability of PTEN and GAPDH mRNA in IncARSR stably overexpressed or deleted SMMC-7721 cells over time was measured by qPCR relative to time 0 after blocking novel RNA synthesis with α-amanitin (50 mM) and normalized to 18S rRNA (a product of RNA polymerase I that is unchanged by α-amanitin). (F, G) PTEN mRNA levels in IncARSR stably overexpressed or deleted SMMC-7721 cells. Data are shown as mean ± SD from at least three independent experiments. **P < 0.01 by Student’s t-test.

Fig. 5. The negative correlation between PTEN and IncARSR expression in HCC. (A) PTEN mRNA levels in 92 pairs of HCC tissues and adjacent noncancerous liver tissues were quantified by qPCR. Data are shown as median with interquartile range. \( P < 0.0001 \) by Wilcoxon signed-rank test. (B) The negative correlation between PTEN and IncARSR expression in these HCC tissues. \( r = -0.7381 \), \( P < 0.0001 \) by Pearson correlation analysis.
Fig. 6. The roles of lncARSR on PTEN-PI3K/Akt pathway. (A) The protein levels of PTEN, p-AKT, and AKT in lncARSR stably overexpressed and control SMMC-7721 cells were measured using Western blot. (B) The protein levels of PTEN, p-AKT, and AKT in lncARSR stably depleted and control SMMC-7721 cells were measured using Western blot. Data are shown as mean ± SD from at least three independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001 by Student’s t-test.

Fig. 7. PTEN-PI3K/Akt pathway is responsible for lncARSR-mediated doxorubicin sensitivity of HCC cells. (A) lncARSR overexpressed and control SMMC-7721 cells were treated with a series dose of doxorubicin (0, 0.3, 0.6, 1.2, 2.4 μM) with or without LY294002 (10 μM) for 24 h. The cell viability was measured using CCK-8 assays, and the data was normalized to the viability of cells with no doxorubicin treatment. (B) lncARSR overexpressed and control SMMC-7721 cells were treated with 0.3 μM doxorubicin with or without LY294002 (10 μM) for 24 h. The cell apoptosis was detected using TUNEL staining. Scale bars, 100 μm. (C) PTEN mRNA levels in lncARSR and PTEN simultaneously depleted or control SMMC-7721 cells. (D) lncARSR and PTEN simultaneously depleted or control SMMC-7721 cells were treated with a series dose of doxorubicin (0, 0.2, 0.4, 0.8, 1.2 μM) for 24 h. The cell viability was measured using CCK-8 assays, and the data was normalized to the viability of cells with no doxorubicin treatment. (E) lncARSR and PTEN simultaneously depleted or control SMMC-7721 cells were treated with 0.2 μM doxorubicin for 24 h. The cell apoptosis was detected using TUNEL staining. Scale bars, 100 μm. Data are shown as mean ± SD from at least three independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001 by Student’s t-test.
IncARSR, which is upregulated in HCC and associated with large tumor size, advanced BCLC stage and poor prognosis, plays important roles in doxorubicin resistance of HCC cells. Overexpression of IncARSR induces doxorubicin resistance in vitro and in vivo, and while knockdown of IncARSR enhances doxorubicin sensitivity of HCC cells in vitro and in vivo. These data implied that the combination of targeting IncARSR and doxorubicin would be a promising therapeutic strategy for HCC. The effects of IncARSR on chemo-resistance of HCC to other drugs, such as 5-fluorouracil, cisplatin and etoposide, and also sensitivity of HCC to receptor tyrosine kinase inhibitor sorafenib need further investigation.

In this study, we also identified a new action mechanism for IncARSR. Except the reported binding to microRNAs and proteins, we found that IncARSR directly physically associates with PTEN mRNA, promotes PTEN mRNA degradation, decreases PTEN expression, and activates PI3K-Akt pathway. The negative correlation between IncARSR and PTEN expression in HCC tissues supported the regulation of PTEN by IncARSR. Furthermore, PI3K/Akt pathway inhibitor could abolish the effects of IncARSR on doxorubicin resistance of HCC cells, and while depletion of PTEN also reverses the effects of IncARSR knockdown on doxorubicin resistance of HCC cells. These functional experiments further support the regulation axis between IncARSR, PTEN, and PI3K-Akt pathway. The detailed mechanisms through which IncARSR promotes PTEN mRNA degradation need further exploration. IncRNA SNHG5 stabilizes its interaction transcript SPATS2 via blocking the degradation of SPATS2 by STAUL [Damas et al., 2016]. BACE1-AS increases BACE1 mRNA stability via preventing miRNA-induced degradation of BACE1 [Faghihi et al., 2008, 2010]. The RNA duplex formed between two transcripts may be processed and degraded by DICER1 [Vermeulen et al., 2005; Ling et al., 2016]. Therefore, whether DICER1 or other proteins and miRNAs participate in the effects of IncARSR on PTEN mRNA degradation need further investigation. Nevertheless, this study demonstrated the diversity and complex of the functions and action mechanisms of IncRNAs in cancers.

In conclusion, we found that upregulated IncARSR promotes doxorubicin resistance in HCC via modulating PTEN-PI3K/Akt pathway. Our data suggested that IncARSR would be a promising prognostic biomarker for HCC, and a potential therapeutic target for HCC chemo-resistance.

REFERENCES


