Research Article

Identification of Four Oxidative Stress-Responsive MicroRNAs, miR-34a-5p, miR-1915-3p, miR-638, and miR-150-3p, in Hepatocellular Carcinoma

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Increasing evidence suggests that oxidative stress plays an essential role during carcinogenesis. However, the underlying mechanism between oxidative stress and carcinogenesis remains unknown. Recently, microRNAs (miRNAs) are revealed to be involved in oxidative stress response and carcinogenesis. This study aims to identify miRNAs in hepatocellular carcinoma (HCC) cells which might involve in oxidative stress response. An integrated analysis of miRNA expression signature was performed by employing robust rank aggregation (RRA) method, and four miRNAs (miR-34a-5p, miR-1915-3p, miR-638, and miR-150-3p) were identified as the oxidative stress-responsive miRNAs. Pathway enrichment analysis suggested that these four miRNAs played an important role in antiapoptosis process. Our data also revealed miR-34a-5p and miR-1915-3p, but not miR-150-3p and miR-638, were regulated by p53 in HCC cell lines under oxidative stress. In addition, clinical investigation revealed that these four miRNAs might be involved in oxidative stress response by targeting oxidative stress-related genes in HCC tissues. Kaplan-Meier analysis showed that these four miRNAs were associated with patients' overall survival. In conclusion, we identified four oxidative stress-responsive miRNAs, which were regulated by p53-dependent (miR-34a-5p and miR-1915-3p) and p53-independent pathway (miR-150-3p and miR-638). These four miRNAs may offer new strategy for HCC diagnosis and prognosis.

1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third cause of cancer-related mortality worldwide [1]. It is established that hepatitis virus infection, as well as environmental carcinogens such as aflatoxin or chemical carcinogens, is associated with the development of HCC [2]. Although the carcinogenic mechanism of above risk factors varies, the common pathological process affected by those risk factors is hepatic chronic inflammation. Recruitment of inflammatory cells in hepatic environment and chemical mediator release, such as cytokines, chemokines, and reactive oxygen species (ROS), are considered to play a vital pathogenic role during hepatic carcinogenesis [3].

ROS are a group of chemically reactive molecules containing oxygen, which are mainly derived from cellular oxidative metabolism and play essential roles in the regulation of multiple cellular processes. During the development of many diseases, including malignant diseases, increasing ROS levels might lead to the imbalance of the pro-oxidant/antioxidant equilibrium and subsequently induce changes of intracellular molecules, including lipids, proteins, and nuclear acids [4]. Thus, the exploration of the intracellular molecules responsible for oxidative stress might enrich our understanding of molecular hepatic carcinogenesis.
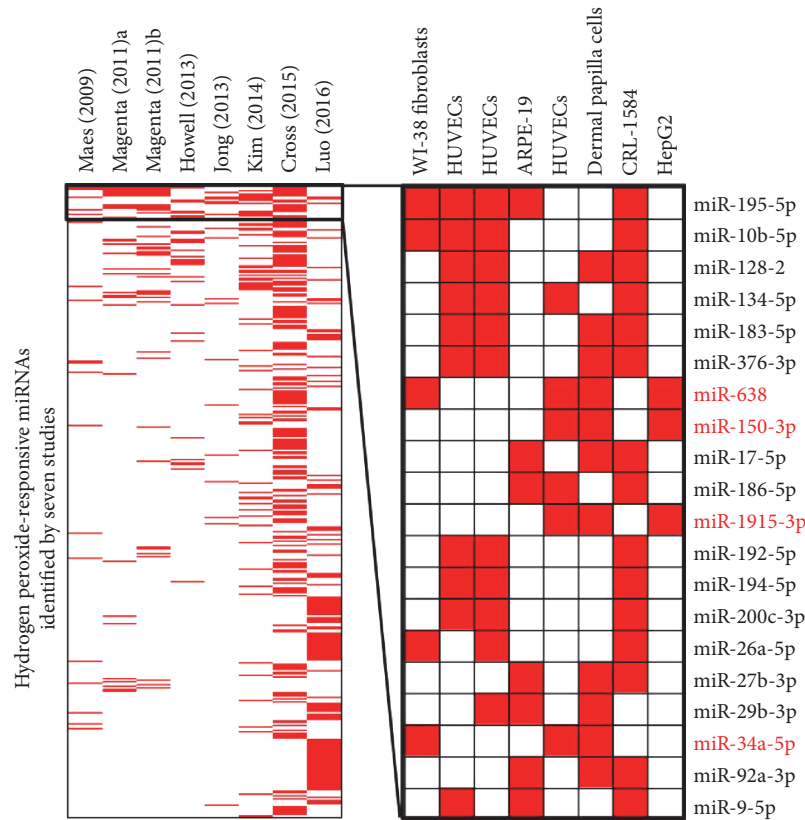
FIGURE 1: Heatmap of miRNAs reported by nine miRnome profiling studies. One selected miRNA was presented as red or white according to whether it was report by one study or not.

Recently, accumulating evidences suggest that a series of small noncoding RNAs (microRNA) can be induced by oxidative stress. Several studies have examined the changes of the microRNA (miRNAs) expression profiles in varying cells upon treatment with hydrogen peroxide (H_2O_2) [5–14]. Unfortunately, inconsistent conclusion was made from those miRNome profiling studies. Confounding factors may include employment of different cell origins, various detection platforms, and application of different statistical methods.

To overcome those limitations, in the present study, we integrated these published relevant studies and performed a meta-analysis applying the rank aggregation method. We identified four common oxidative stress-responsive microRNAs in H_2O_2-treated cells. Moreover, we also evaluated the association between those oxidative stress-responsive microRNAs and p53, a key oxidative stress-responsive mediator in HCC cell lines. Finally, we validated the expression of identified miRNAs and their target genes in HCC tissues.

2. Materials and Methods

2.1. Literature Search and Inclusion Criteria.
We performed a literature search in PubMed, Embase, and Web of Knowledge databases using search term combination of (miRNA* or
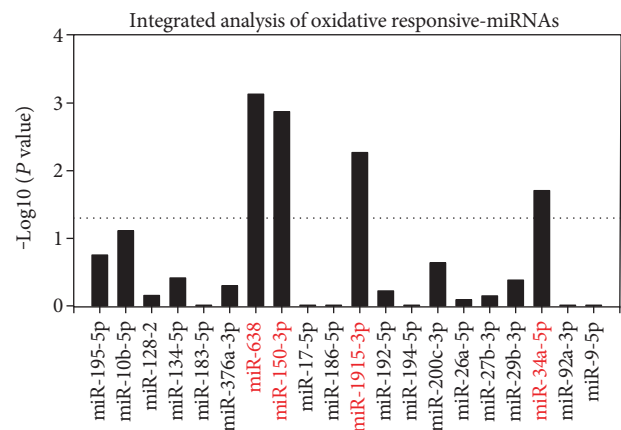

FIGURE 2: Integrated analysis of oxidative responsive-miRNAs by RRA method. The column was presented according to $-\text{Log}_{10}$ (P value) of each miRNA which was calculated by RRA method.

microRNA* or mir-*) and profil* and (oxidati* or hydrogen peroxide) and (cell* or cell line*). We firstly screened all abstracts and selected potential abstracts for further full text evaluation. Only the original experimental studies that explored miRNA profile using a high-throughput miRNA expression profiling methods such as second-generation sequencing, polymerase chain reaction (PCR),
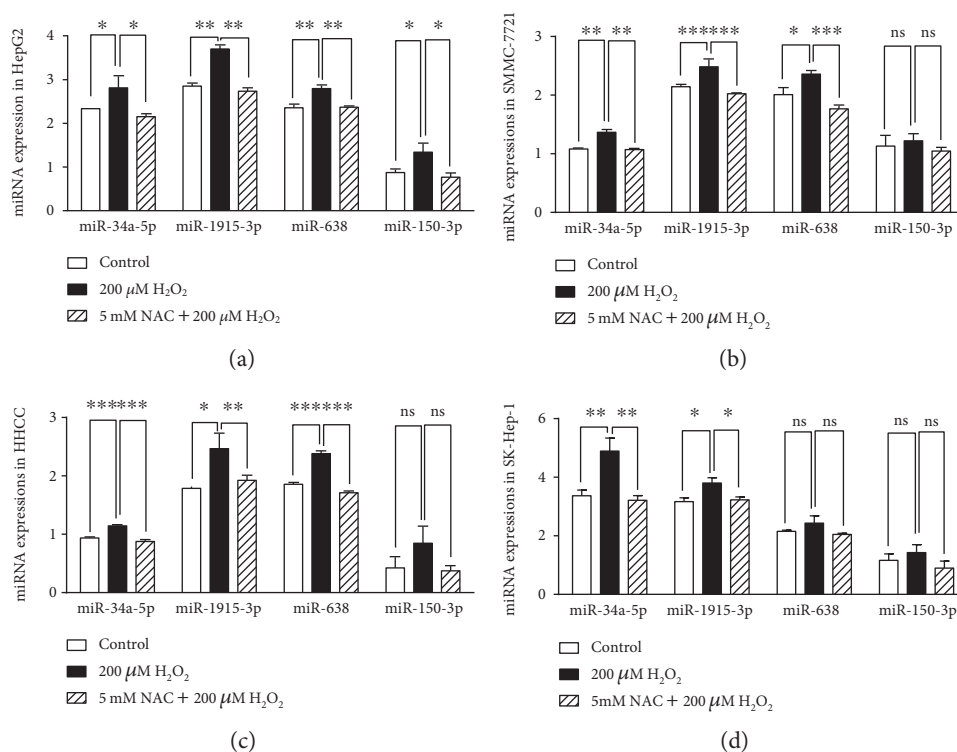
FIGURE 3: Expression of four oxidative stress-responsive miRNAs in different HCC cell lines. The expressions of miR-34a-5p, miR-1915-3p, miR-638, and miR-150-3p were measured by qRT-PCR method in HepG2 (a), SMMC-7721 (b), HHCC (c), and SK-Hep-1 (d). All HCC cell lines were treated with saline, $200\,\mu M$ H_2O_2 or $5\,mM$ NAC + $200\,\mu M$ H_2O_2, respectively. All these experiments were conducted in triplicate. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ns, no significant.

or microarray-based high-throughput methods in H_2O_2-treated cells were included.

2.2. Data Extraction and Rank Aggregation Analysis. Rank lists of up- or downregulated miRNAs were extracted from the included studies. All included miRNA names were firstly standardized using miRBase (release 21.0). Rank aggregation method was implemented using an R package "Robust Rank Aggreg." This method analyzed miRNAs that are ranked consistently better than expected and assigns a P value for each miRNA.

2.3. Pathway Enrichment Analysis. We firstly collected validated targets of each miRNA using miRTarBase database (http://mirtarbase.mbc.nctu.edu.tw/). Gene ontology (GO) biological process enrichment of those validated targets was performed using Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.8 (https://david.ncifcrf.gov/). Fisher's exact test was used to identify the significant pathway terms. Heatmap was presented by log-transformed P value. Moreover, the combinatorial pathway enrichment analysis of multiple miRNAs was analyzed using "miRPath" algorithm (http://www.microrna.gr/miRPathv2).

2.4. miRNA-Gene Interaction Network Analysis. We firstly performed target prediction by using Targetscan website (http://www.targetscan.org/), and only cumulative-weighted context++ score< −0.4 was selected for further analysis.

The interaction between miRNA and their predicted target networks was constructed using the Cytoscape software. In the validation group, the miRNA-gene interaction was presented based on their Pearson's correlation coefficient.

2.5. HCC Cell Line Culture. Four TP53 wild-type HCC cell lines, including HepG2, SMMC-7721, HHCC, and SK-Hep-1, were selected for experimental validation. All cell lines were cultured using Dulbecco minimum essential medium (DMEM) supplemented with heat-inactivated 10% fetal bovine serum (FBS), 100 U/ml penicillin, and $100\,\mu g$/ml streptomycin. Cells were incubated at 37°C and 5% CO_2 in a HF100 chamber (Heal Force, Hong Kong, China).

2.6. Pro- and Antioxidant Treatment. For pro-oxidant treatment, HCC cell lines were firstly synchronized by serum starvation overnight and then treated with $200\,\mu M$ of H_2O_2 for 24 h. For antioxidant treatment, cells were preincubated with N-acetylcysteine (NAC, 5 mM) for 6 hours before exposure to H_2O_2. Following treatment, cells were harvested for further analysis.

2.7. Transfection of siRNA. RNA interference mediated by duplexes of 21-nucleotide RNA was performed in HCC cell lines. siRNA-TP53 (5$'$-CUGGAAGACUCCAGUG GUA-3) and control-siRNA were synthesized by Gene-Pharma (Shanghai, China). Transfection of siRNA
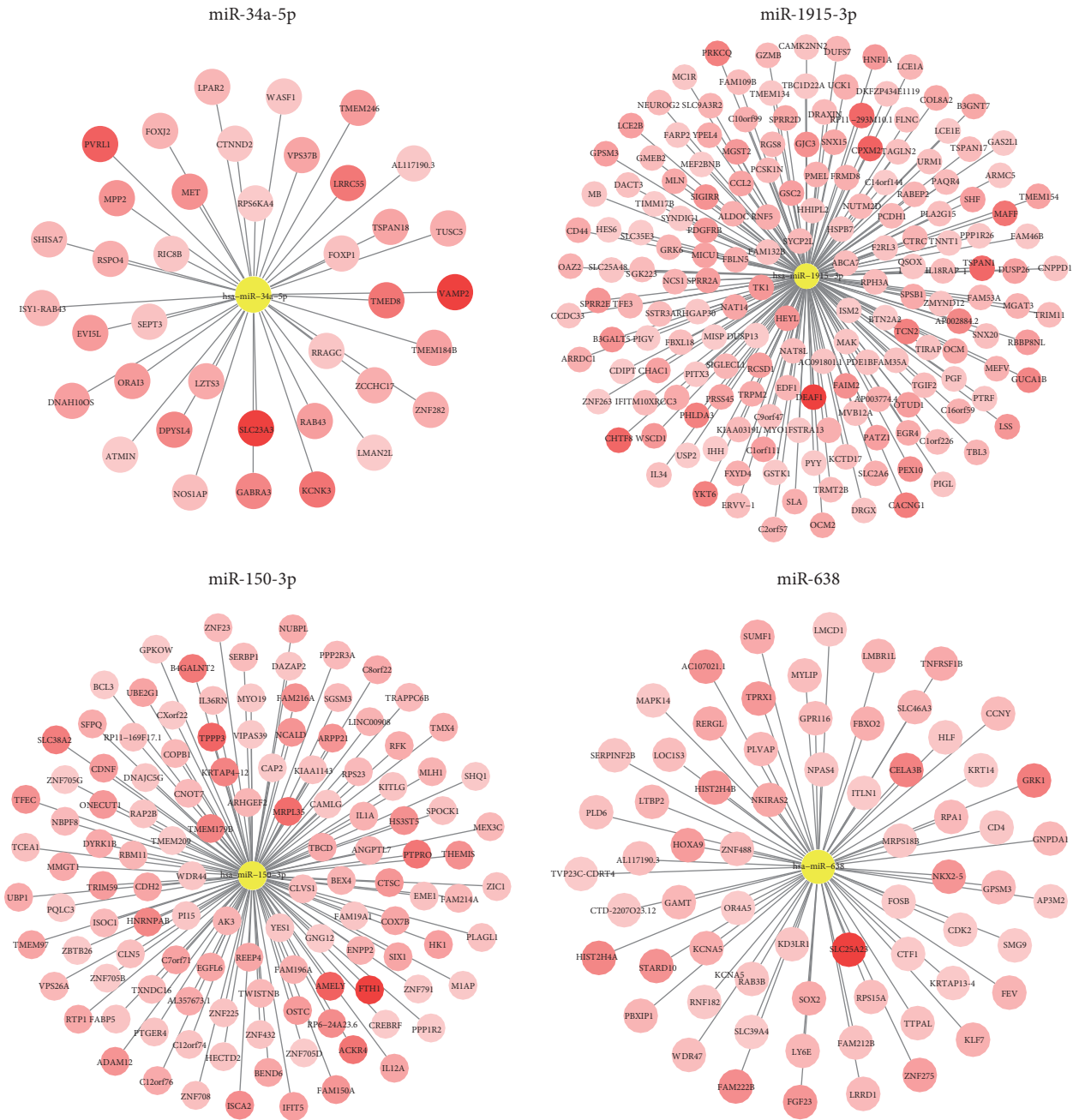
Figure 4: The interaction network between four miRNAs and their predicted targets.

(100 nM) was carried out with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocols.

2.8. Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR). Total RNA was extracted from cultured cells using Trizol reagent (Invitrogen, Carlsbad, CA, USA). qRT-PCR was performed using the SYBR® PrimeScript™ miRNA RT-PCR Kit and SYBR Premix Ex Taq™ (TaKaRa Biotechnology, Dalian, China). All primers of selective miRNAs were also synthesized by TaKaRa. The miRNA expression was assayed in triplicate and normalized to U6.

The relative miRNA expression was calculated using the comparative-Ct ($\triangle\triangle$Ct) method.

2.9. Public Datasets for Validation. To validate the expression of miRNAs and their targets in HCC tissues, we used public datasets containing 100 paired HCC tissue samples (GSE62007 and GSE62043). GSE62007 dataset was conducted on miRXplore Microarray platform, and GSE62043 dataset was conducted on Agilent-014850 Whole Human Genome Microarray platform. For hepatocellular carcinoma, The Cancer Genome Atlas (TCGA) dataset were used to explore the prognosis
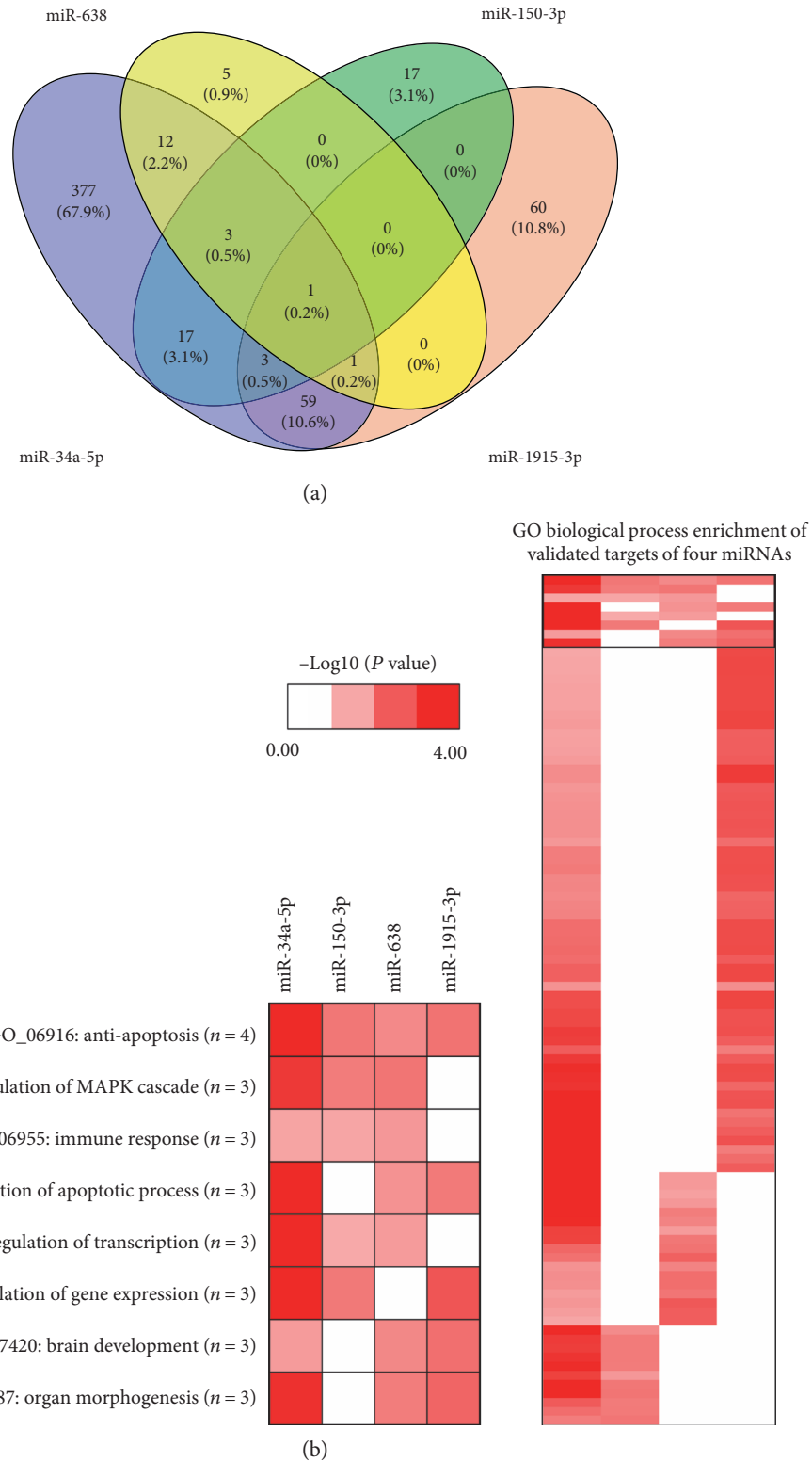
(a)

(b)

FIGURE 5: Pathway enrichment analysis of four oxidative stress-responsive miRNAs. (a) Venn diagram of pathways of four oxidative stress-responsive miRNAs. (b) Heatmap of pathways of four oxidative stress-responsive miRNAs. Range of colors (white to deep red) shows the $-\text{Log}_{10}$ (P value of pathway enrichment analysis) (low to high).

values of four miRNAs in HCC. The data were obtained from SurvMicro web tool (http://bioinformatica.mty.itesm. mx/SurvMicro).

2.10. Statistical Analysis. All data in this study were analyzed by SPSS 11.0 software (SPSS Inc., Chicago, IL, USA) and expressed as mean ± standard error of measurement (SEM).

Analysis of continuous variables was performed using a Student t-test. Expression correlation between variables was tested by Pearson's correlation analysis. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Identification of Oxidative Stress-Responsive miRNAs. A total of 11 studies from 10 published articles were screened [5–14], and 8 studies were based on human cells [6, 7, 10–14], which were selected for further analysis. In summary, 504 aberrantly expressed miRNAs were recorded, including 404 upregulated and 99 downregulated miRNAs. Among them, twenty upregulated miRNAs were reported by more than three studies (Figure 1). By applying a rank aggregation analysis, we identified four oxidative stress-responsive miRNAs which were significant upregulated under H_2O_2 treatment, including miR-638, miR-150-3p, miR-1915-3p, and miR-34a-5p (Figure 2).

3.2. Validation of Oxidative Stress-Responsive miRNAs in HCC Cell Lines. To further explore the expression changes of identified miRNAs in oxidative stress response in HCC, we treated four *TP53* wild-type HCC cell lines with $200\,\mu M$ H_2O_2 (the most widely used concertation) for 24 h and measured expression levels of four miRNAs (miR-34a-5p, miR-1915-3p, miR-638, and miR-150-3p). Our data revealed that the expressions of four miRNAs were all significantly increased after H_2O_2 treatment. By contrast, when preincubation with 5 mM NAC for 6 h, the upregulated expressions of miR-34a-5p, miR-1915-3p, miR-638, and miR-150-3p caused by H_2O_2 treatment were abrogated (Figure 3). The above results provide evidence that the expressions of miR-34a-5p, miR-1915-3p, miR-638, and miR-150-3p were tightly associated with intracellular oxidative stress status in HCC cell lines.

3.3. Function Prediction of Oxidative Stress-Responsive miRNAs. We firstly performed target prediction by using Targetscan web tool, and the miRNA-target interaction was visualized in Figure 4. To further evaluate biological functions of four miRNAs, we selected their experimental validated targets for GO pathway enrichment analysis. The number of enriched GO biological process of each miRNA ranged from 22 to 474 (Figure 5(a)). By conducting a heatmap of P values derived from Fisher's exact test, four miRNAs were shown to be all closely related to antiapoptosis pathways (Figure 5(b)). Furthermore, combinatorial pathway enrichment analysis revealed that those four oxidative stress-responsive miRNAs were related to p53 signaling pathway (Table 1). Previous studies had clearly demonstrated the closely association between p53 signaling pathway and oxidative stress [15, 16], suggesting a critical role of four miRNAs (miR-34a-5p, miR-1915-3p, miR-638, and miR-150-3p) in oxidative stress response.

3.4. miR-34a-5p and miR-1915-3p Are Regulated by p53 under Oxidative Stress. Since our data suggested an association between oxidative stress-responsive miRNAs and p53

TABLE 1: The signaling pathway enrichments of validated targets of four miRNAs.

KEGG signaling pathways	P value
KEGG_04115: p53 signaling pathway	$2.24E-10$
KEGG_04310: Wnt signaling pathway	$4.83E-09$
KEGG_04330: notch signaling pathway	$1.34E-06$
KEGG_04630: Jak-STAT signaling pathway	$3.83E-04$
KEGG_04722: neurotrophin signaling pathway	$1.73E-03$
KEGG_04340: hedgehog signaling pathway	$1.89E-03$
KEGG_04010: MAPK signaling pathway	$3.33E-03$
KEGG_04370: VEGF signaling pathway	$4.06E-03$
KEGG_04350: TGF-beta signaling pathway	$4.98E-03$
KEGG_04660: T cell receptor signaling pathway	$9.59E-03$
KEGG_04150: mTOR signaling pathway	$2.14E-02$
KEGG_04920: adipocytokine signaling pathway	$3.27E-02$
KEGG_03320: PPAR signaling pathway	$3.36E-02$

signaling pathway, we wondered whether the expressions of these miRNAs in oxidative stress response are regulated by p53. Evidences from our [17, 18] and other groups [19] suggested that p53 was upregulated in response to oxidative stress in HCC. Thus, by employing four HCC cell line data, we quantified the correlation coefficient between p53 and four oxidative stress-responsive miRNAs expression. We found that p53 gene (*TP53*) was statistically positively correlated to the expressions of miR-34a-5p and miR-1915-3p ($P < 0.05$), but not miR-638 and miR-150-3p ($P > 0.05$) (Figure 6), indicating that p53 might regulate the miR-34a-5p and miR-1915-3p function in oxidative stress response. To further verify this hypothesis, we then examined the miR-34a-5p and miR-1915-3p expressions in siRNA-*TP53*-transfected HCC cells. As shown in Figures 7(a) and 7(c), the miR-34a-5p and miR-1915-3p expression levels were significantly decreased by knocking down of *TP53* (all $P < 0.05$). Furthermore, knocking down of *TP53* was shown to be able to attenuate the increased expression of miR-34a-5p and miR-1915-3p induced by H_2O_2 treatment, providing solid evidence for the regulatory roles of p53 in miR-34a-5p and miR-1915-3p in oxidative stress response (Figures 7(b) and 7(d)).

3.5. Clinical Values of Four Oxidative Stress-Responsive miRNAs in HCC. To gain insight into clinical values of four oxidative stress-responsive miRNAs in HCC, we firstly obtained 4 miRNAs and 90 oxidative stress-related gene profiling data based on 100 paired HCC samples (GSE62007 and GSE62043) from Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) (Figure 8(a)). By calculating the expression correlations between each miRNA and oxidative stress-related gene, we conducted a miRNA-gene interaction network (Figure 8(b)). Our data showed that miR-150-3p and miR-1915-3p were negatively correlated with more than half of oxidative stress-related genes (54/90 and 50/90, resp.). miR-34a-5p was negatively correlated with 44 out of 90 oxidative stress-
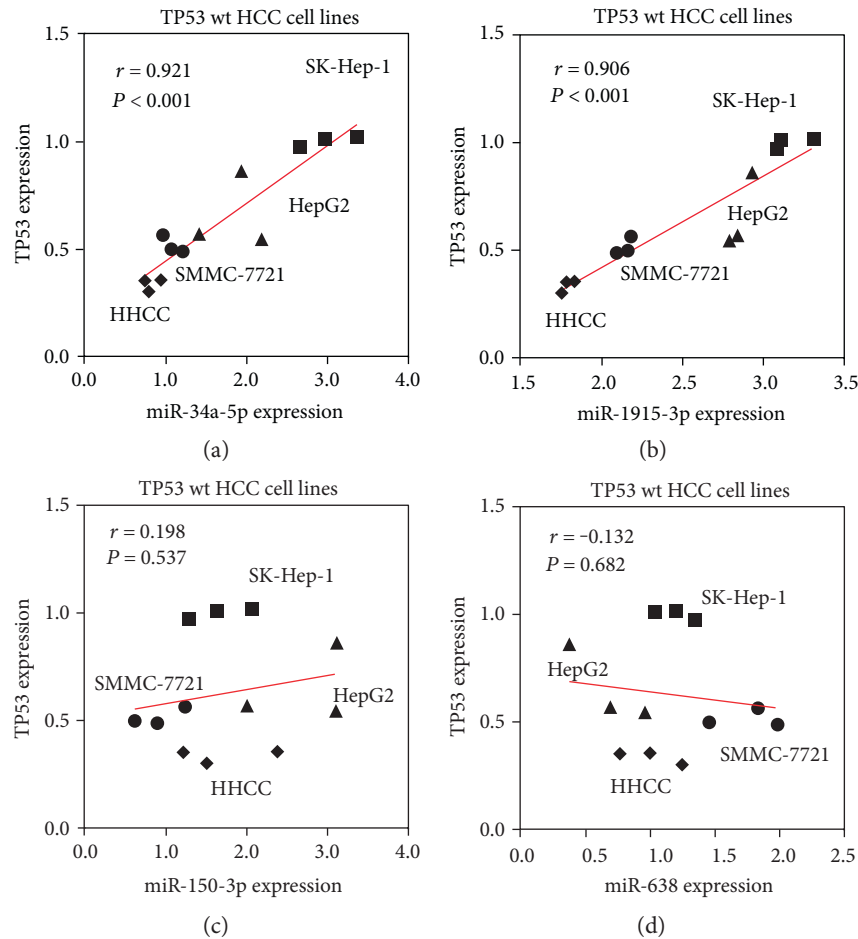
FIGURE 6: The expression correlation between four miRNAs and *TP53*. (a) miR-34a-5p, (b) miR-1915-3p, (c) miR-150-3p, and (d) miR-638.

related genes. Meanwhile, miR-638 was only negatively correlated with the least number of oxidative stress-related genes (38/90), which was partially consistent with previous study [20]. Moreover, tissue expression pattern also validated the positively correlation between p53 and miR-34a/miR-1915-3p with correlation coefficients of 0.13 and 0.16, respectively.

Next, we explored the prognosis values of four oxidative stress-responsive miRNAs in HCC patients. We obtained HCC TCGA data by using SurvMicro web tool (http://bioinformatica.mty.itesm.mx/SurvMicro) and conducted Kaplan-Meier analysis of HCC patients with different miRNA expression levels. We found that HCC patients with lower miR-34a and miR-1915-3p expression levels had shorter overall survival time, although we did not get a statistically significant P value which might due to a small sample size (Figures 9(a) and 9(b)). Similarly, we also found that miR-638 and miR-150-3p could predict HCC patients' survival time, with a P value of 0.042 (Figure 9(c)). It should be pointed out that the expression level of miR-638 in high-risk group was slightly higher than that in low risk group, with a nonsignificant P value (Figure 9(d)), which may warrant future larger study to confirm or refuse it.

4. Discussion

For decades, oxidative stress has been reported to impact carcinogenesis. However, the underlying interaction among oxidative stress and carcinogenesis remains unknown. Recently, increasing evidence suggested that some intracellular miRNAs play critical roles in oxidative stress response and carcinogenesis. Therefore, in the current study, we aim to identify several oxidative stress-responsive miRNAs which might involve in liver carcinogenesis. We firstly performed a comprehensive analysis by employing a recently published rank aggregation analysis method [21], to identify oxidative stress-responsive miRNAs. Based on miRNA profiling data, four miRNAs (miR-34a-5p, miR-1915-3p, miR-638, and miR-150-3p) were identified as the common upregulated miRNAs under oxidative stress. Next, we also validated these four oxidative stress-responsive miRNAs in four different HCC cell lines. Specifically, we found that the upregulation of miR-34a-5p and miR-1915-3p, but not miR-638 and miR-150-3p, was depended on p53 status in oxidative stress (Figure 10). Finally, our data also provide clinical evidences for the possible negative regulation of oxidative stress-related genes by these four miR-NAs in HCC tissues. We found that the expression levels
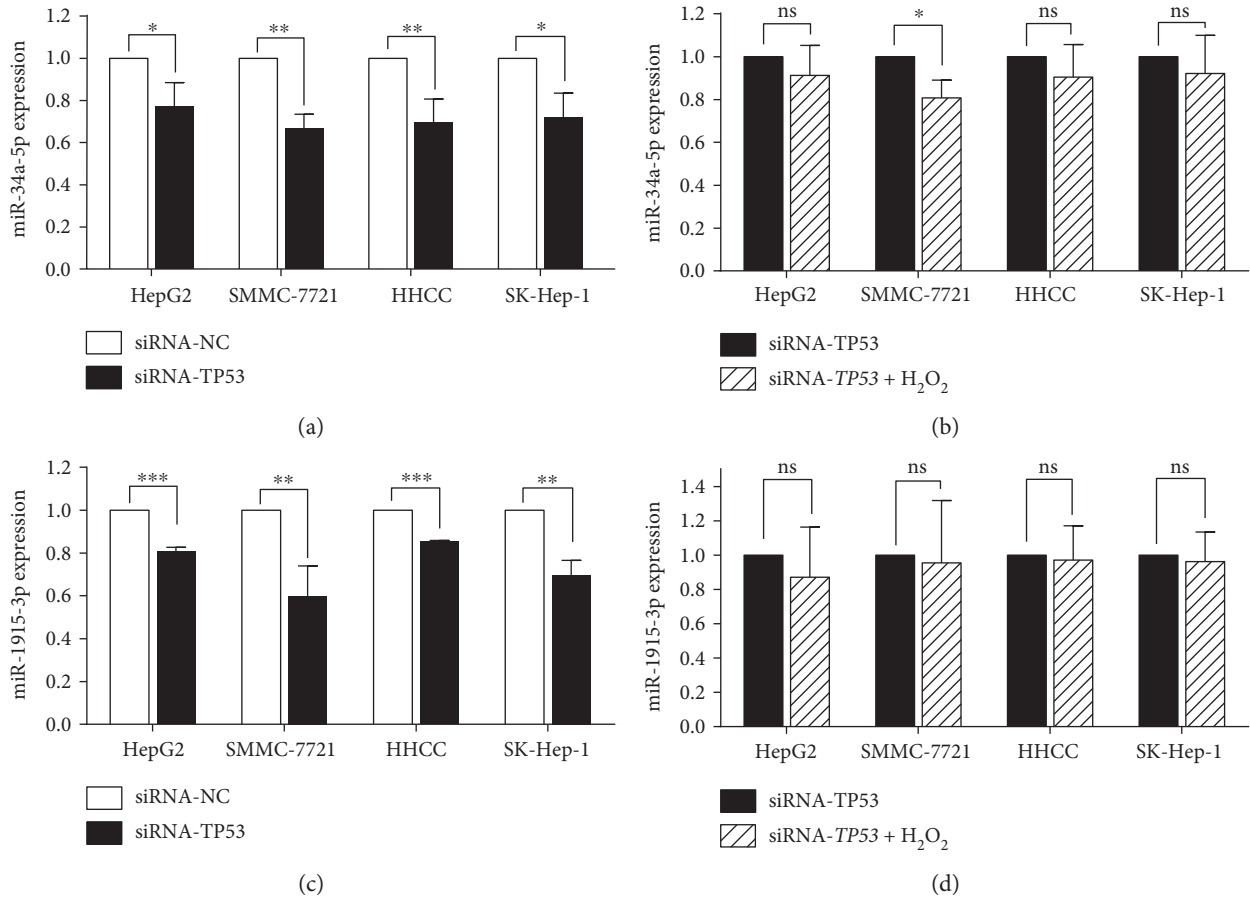
(a)

(b)

(c)

(d)

FIGURE 7: miR-1915-3p and miR-34a-5p are regulated by p53. (a, c) After transfection of siRNA-*TP53*, the expression of miR-34a-5p and miR-1915-3p was analyzed by qRT-PCR. (b, d) After transfection of siRNA-*TP53*, the expression of miR-34a-5p and miR-1915-3p was measured with or without H_2O_2 treatment. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ns, no significant.

of those four miRNAs were associated with overall survival time in HCC patients.

miR-34a-5p is one of the most explored miRNAs in carcinogenesis [22]. Although comparatively low levels of miR-34a-5p expression were reported in several cancers [23, 24], discrepancies emerged in HCC. Researchers found that the expression of miR-34a was downregulated during methyl-deficient diet-induced liver carcinogenesis [25]. By contrast, in a chemical-induced HCC model, miR-34a-5p was found to be upregulated [26]. We speculate that miR-34a-5p might be regulated by different mechanisms during liver carcinogenesis. It has been widely accepted that chemotherapeutic agents exhibited anticancer effects by producing ROS in the target tissue. We observed the ROS generation in HCC cells with cisplatin treatment [17]. Bai et al. also found that miR-34a-5p suppressed mitochondrial antioxidative enzymes with a concomitant increase in intracellular ROS level [27]. All above evidences suggested the miR-34-5p expression might be related to ROS level in HCC cells. In the present study, by conducting an oxidative stress model in four HCC cell lines, we confirmed that miR-34a-5p was related to intracellular oxidative stress status. Furthermore, we also found miR-34a-5p was involved in the p53-

dependent pathway, which was consistent with previous results [22, 28].

miR-1915-3p was another dysregulated miRNA in various types of cancer, such as prostate cancer, renal cell carcinoma, breast cancer, lung cancer, and colorectal cancer [29–33]. However, the function of miR-1915-3p remains unknown in HCC. The present study revealed for the first time that miR-1915-3p was upregulated by oxidative stress, depending on p53 status in HCC. Recently, a study based on colorectal cancer cells also confirmed our results [32]. The authors [32] reported that miR-1915-3p was involved in response to chemotherapeutic drug-induced DNA damage by targeting *Bcl-2*, the latter of which was an important regulator of oxidative stress [34]. Additionally, they also found that p53 induced the processing of pri-miR-1915-3p to pre-miR-1915-3p, which promote overexpression of mature miR-1915-3p in. These data suggested that, differing from miR-34a-5p, miR-1915-3p expression was regulated by p53 in a transcription-independent way. When focusing on the genes potentially affect oxidative stress, we found that many oxidative stress-related genes might be potential targets of miR-1915-3p. These evidences suggest that by targeting oxidative stress-related genes, miR-
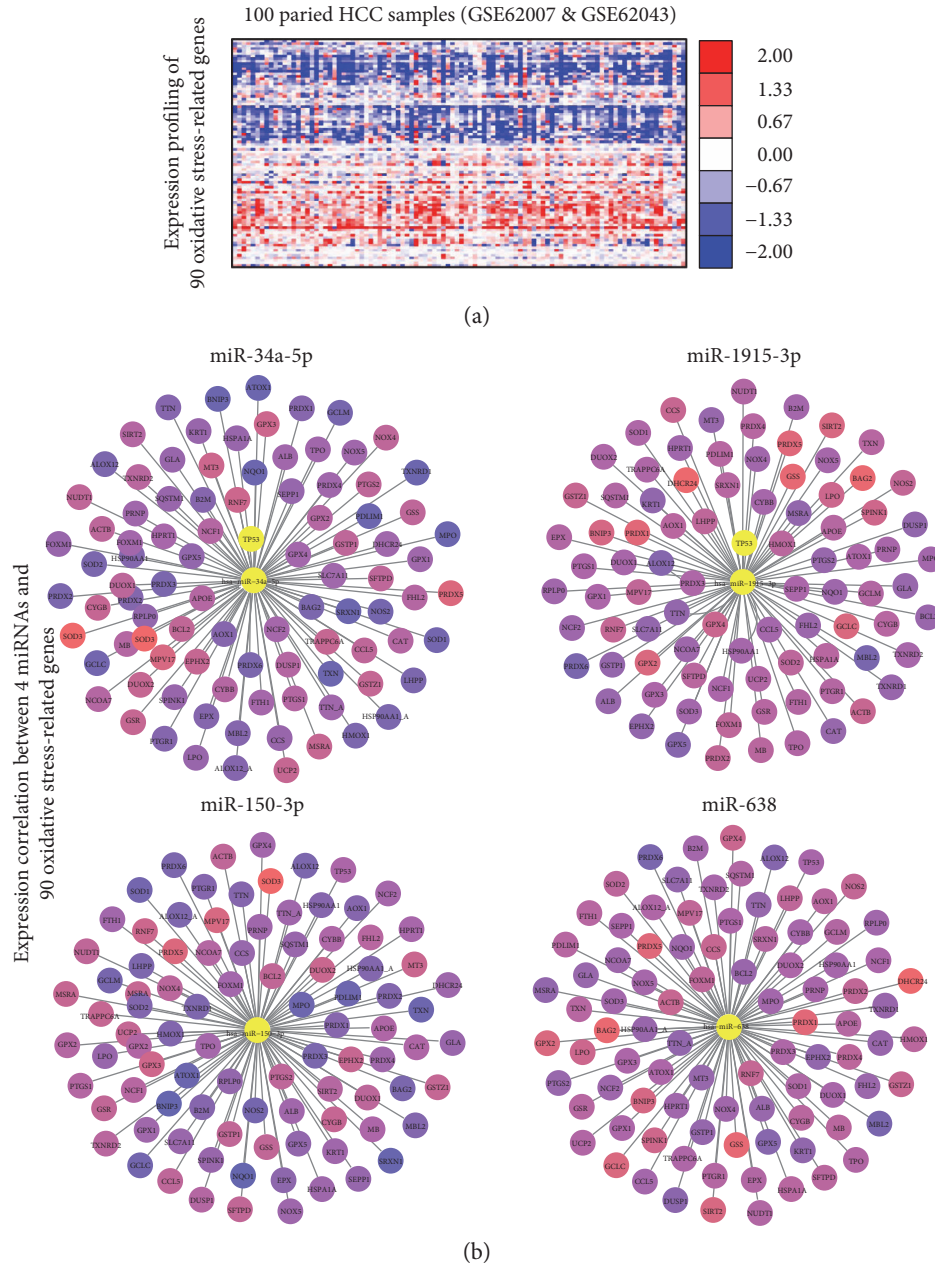
FIGURE 8: Four miRNAs and oxidative stress-related genes in HCC. (a) Heatmap of 90 oxidative stress-related gene expressions in HCC tissues with different miR-1915-3p status. (b) The expression correlation between four miRNAs and 90 oxidative stress-related genes. Range of colors (blue to red) was represented according to Pearson's correlation coefficient (low to high).

1915-3p might possess a significant role in the regulation of oxidative stress response.

miR-638 was previously reported to be associated with cellular senescence [6] and DNA damage [35]. In 2013, Christenson et al. [20] screened the gene expression in miR-638-inhibited fibroblasts and suggested that miR-638 might involve in oxidative stress response. In the present study, we, for the first time, identified miR-638 as an oxidative stress-induced miRNA in HCC cell lines. We found that the elevated expression of miR-638 was not induced by p53. However, the regulation mechanisms of miR-638 during oxidative stress have hardly been investigated. Tay

et al. found that miR-638 was encoded by Dnm2 locus and demonstrated that both two components, miR-638 and its host gene Dnm2, were overexpressed in tumorigenesis [36]. Moreover, it was also reported that Dnm2 could be upregulated by radiation-induced oxidative stress [37]. Therefore, we hypothesized that oxidative stress might promote transcription of host gene Dnm2 and miR-638 was cooperatively upregulated. Furthermore, our data also showed that HCC patients with high risk had a higher expression level of miR-638 than low risk group. However, the association between miR-638 and cancer prognosis was inconsistent. Some studies showed that the
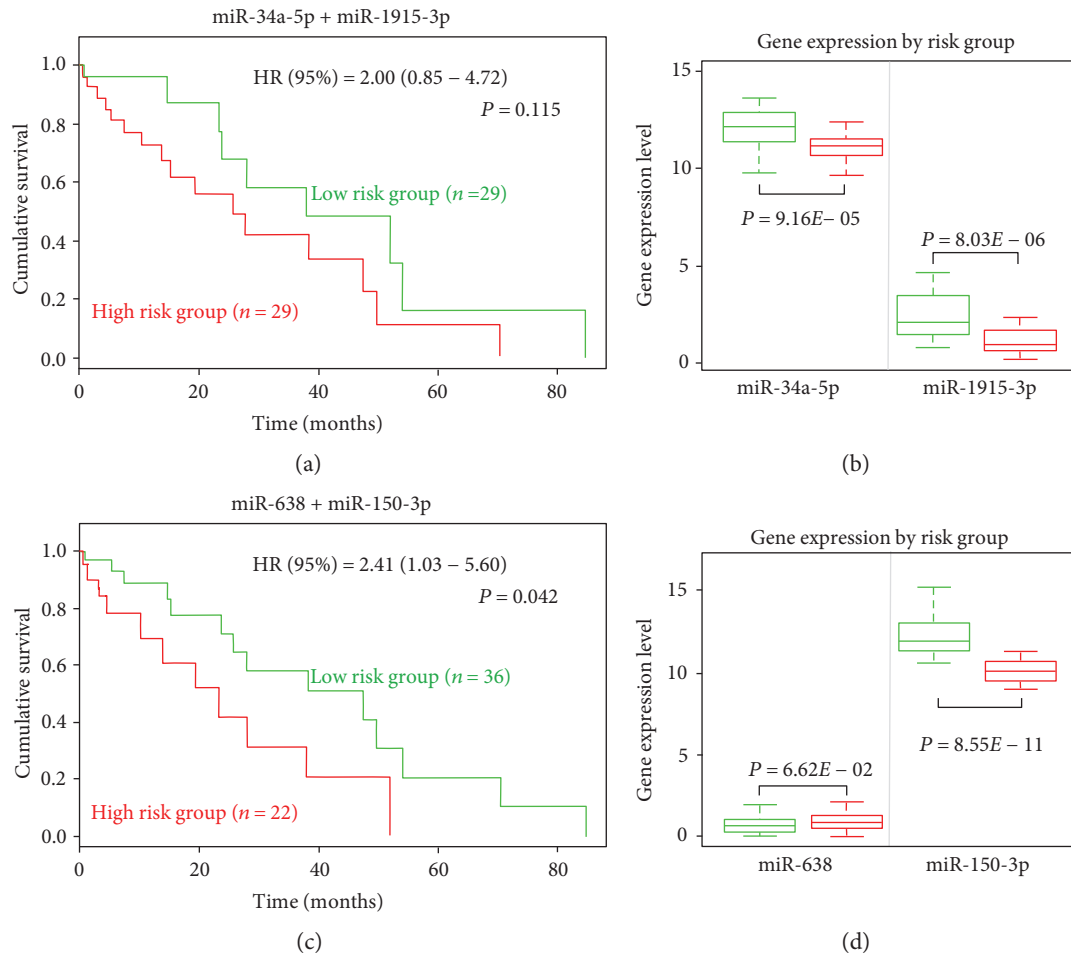
(a)

(b)

(c)

(d)

FIGURE 9: Prognosis values of four miRNAs in HCC. (a) Kaplan-Meier curve of overall survival for patients with different risk groups identified by miR-34a-5p and miR-1915-3p. (b) The expression levels of miR-34a-5p and miR-1915-3p in different risk groups. (c) Kaplan-Meier curve of overall survival for patients with different risk groups identified by miR-150-3p and miR-638. (d) The expression levels of miR-150-3p and miR-638 in different risk groups.
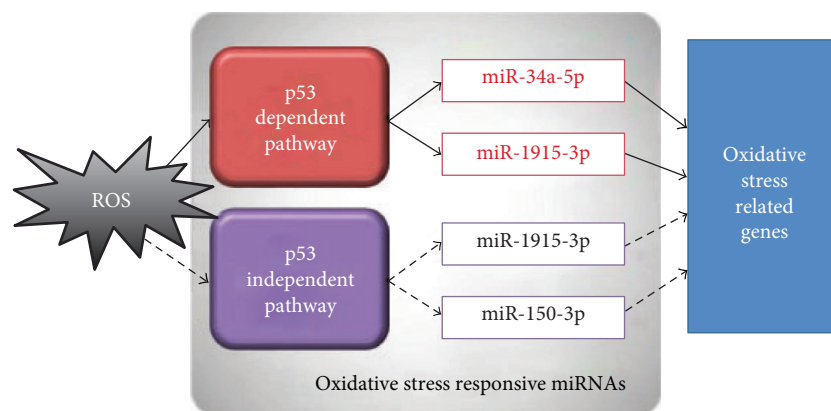

FIGURE 10: Overview of oxidative stress-responsive miRNAs mechanism in HCC. Oxidative stress-induced miRNA by p53-dependent (miR-34a-5p and miR-1915-3p) or p53-independent (miR-638 and miR-150-3p) pathways. All those oxidative stress-responsive miRNAs inhibit translation of their target genes and involve in oxidative stress process.

downregulation of miR-638 was involved in malignant phenotypes of osteosarcoma, gastric carcinoma, and colorectal carcinoma [38–40]. By contrast, Bhattacharya et al. [41] reported that miR-638 promoted melanoma progression.

Besides, Ren et al. [42] also found that miR-638 acts as an oncogene and promotes cell proliferation and metastasis in esophageal squamous cell carcinoma and breast cancer cells. Considering our data did not get a statistically significant

P value, the jury must refrain from drawing a firm conclusion until a well-designed clinical study confirms or refutes the above findings.

Additionally, we also observed that miR-150-3p was induced by oxidative stress and was not depended on p53 overexpression. However, its functions in oxidative response and liver carcinogenesis have yet to be unravelled. It was reported that miR-150-3p could be induced by NF-kB activation [43]. Considering the important roles of NF-kB pathway in oxidative stress response and inflammation [44], investigating the underlying interaction between NF-kB and miR-150-3p will be of particular interest to study the molecular mechanisms of oxidative stress response in liver carcinogenesis.

Taken together, we identified four oxidative stress-responsive miRNAs (miR-34a-5p and miR-1915-3p, but not miR-638 and miR-150-3p) and further demonstrated p53-dependent and p53-independent mechanisms of miRNA expression regulation in HCC. Given a critical role for oxidative stress in carcinogenesis, our present findings have significant clinical implications. These miRNAs might offer new potential strategy for cancer diagnosis and prognosis.

Conflicts of Interest

The authors declare that they have no conflict of interests.

Authors' Contributions

Kai Qu and Chang Liu designed the research and wrote the paper; Yong Wan, Ting Lin, Ruixia Cui, Jingxian Gu, and Xing Zhang participated in the research work; Ting Lin and Xiaohong Xiang collected and analyzed the data; Yong Wan constructed the figures; and Kai Qu drafted and revised the manuscript.

Acknowledgments

References

[1] A. Jemal, F. Bray, M. M. Center, J. Ferlay, E. Ward, and D. Forman, "Global cancer statistics," *CA: A Cancer Journal for Clinicians*, vol. 61, no. 2, pp. 69–90, 2011.

[2] H. B. El-Serag, "Epidemiology of viral hepatitis and hepatocellular carcinoma," *Gastroenterology*, vol. 142, no. 6, article e1261, pp. 1264–1273, 2012.

[3] J. K. Stauffer, A. J. Scarzello, Q. Jiang, and R. H. Wiltrout, "Chronic inflammation, immune escape, and oncogenesis in the liver: a unique neighborhood for novel intersections," *Hepatology*, vol. 56, no. 4, pp. 1567–1574, 2012.

[4] R. Cardin, M. Piciocchi, M. Bortolami et al., "Oxidative damage in the progression of chronic liver disease to hepatocellular carcinoma: an intricate pathway," *World Journal of Gastroenterology*, vol. 20, no. 12, pp. 3078–3086, 2014.

[5] Y. Lin, X. Liu, Y. Cheng, J. Yang, Y. Huo, and C. Zhang, "Involvement of MicroRNAs in hydrogen peroxide-mediated gene regulation and cellular injury response in vascular smooth muscle cells," *The Journal of Biological Chemistry*, vol. 284, no. 12, pp. 7903–7913, 2009.

[6] O. C. Maes, H. Sarojini, and E. Wang, "Stepwise up-regulation of microRNA expression levels from replicating to reversible and irreversible growth arrest states in WI-38 human fibroblasts," *Journal of Cellular Physiology*, vol. 221, no. 1, pp. 109–119, 2009.

[7] A. Magenta, C. Cencioni, P. Fasanaro et al., "miR-200c is upregulated by oxidative stress and induces endothelial cell apoptosis and senescence via ZEB1 inhibition," *Cell Death and Differentiation*, vol. 18, no. 10, pp. 1628–1639, 2011.

[8] B. Mateescu, L. Batista, M. Cardon et al., "miR-141 and miR-200a act on ovarian tumorigenesis by controlling oxidative stress response," *Nature Medicine*, vol. 17, no. 12, pp. 1627–1635, 2011.

[9] S. Xu, R. Zhang, J. Niu et al., "Oxidative stress mediated-alterations of the microRNA expression profile in mouse hippocampal neurons," *International Journal of Molecular Sciences*, vol. 13, no. 12, pp. 16945–16960, 2012.

[10] J. C. Howell, E. Chun, A. N. Farrell et al., "Global microRNA expression profiling: curcumin (diferuloylmethane) alters oxidative stress-responsive microRNAs in human ARPE-19 cells," *Molecular Vision*, vol. 19, pp. 544–560, 2013.

[11] H. L. Jong, M. R. Mustafa, P. M. Vanhoutte, S. AbuBakar, and P. F. Wong, "MicroRNA 299-3p modulates replicative senescence in endothelial cells," *Physiological Genomics*, vol. 45, no. 7, pp. 256–267, 2013.

[12] O. Y. Kim, H. J. Cha, K. J. Ahn, I. S. An, S. An, and S. Bae, "Identification of microRNAs involved in growth arrest and cell death in hydrogen peroxide-treated human dermal papilla cells," *Molecular Medicine Reports*, vol. 10, no. 1, pp. 145–154, 2014.

[13] C. E. Cross, M. F. Tolba, C. M. Rondelli, M. Xu, and S. Z. Abdel-Rahman, "Oxidative stress alters miRNA and gene expression profiles in villous first trimester trophoblasts," *BioMed Research International*, vol. 2015, Article ID 257090, 2015.

[14] Y. W. X. Luo, L. Wang, J. Gao et al., "Identification of micro-RNAs involved in growth arrest and apoptosis in hydrogen peroxide-treated human hepatocellular carcinoma cell line HepG2," *Oxidative Medicine and Cellular Longevity*, vol. 2016, Article ID 7530853, 2016.

[15] J. Pflaum, S. Schlosser, and M. Muller, "p53 family and cellular stress responses in cancer," *Frontiers in Oncology*, vol. 4, p. 285, 2014.

[16] A. V. Budanov, "The role of tumor suppressor p53 in the antioxidant defense and metabolism," *Sub-Cellular Biochemistry*, vol. 85, pp. 337–358, 2014.

[17] K. Qu, T. Lin, Z. Wang et al., "Reactive oxygen species generation is essential for cisplatin-induced accelerated senescence

in hepatocellular carcinoma," *Frontiers of Medicine*, vol. 8, no. 2, pp. 227–235, 2014.

[18] K. Qu, T. Lin, J. Wei et al., "Cisplatin induces cell cycle arrest and senescence via upregulating P53 and P21 expression in HepG2 cells," *Nan Fang Yi Ke da Xue Xue Bao = Journal of Southern Medical University*, vol. 33, no. 9, pp. 1253–1259, 2013.

[19] J. Zhao, J. Chen, B. Lu et al., "TIP30 induces apoptosis under oxidative stress through stabilization of p53 messenger RNA in human hepatocellular carcinoma," *Cancer Research*, vol. 68, no. 11, pp. 4133–4141, 2008.

[20] S. A. Christenson, C. A. Brandsma, J. D. Campbell et al., "miR-638 regulates gene expression networks associated with emphysematous lung destruction," *Genome Medicine*, vol. 5, no. 12, p. 114, 2013.

[21] U. Vosa, R. Kolde, J. Vilo, A. Metspalu, and T. Annilo, "Comprehensive meta-analysis of microRNA expression using a robust rank aggregation approach," *Methods in Molecular Biology (Clifton, NJ)*, vol. 1182, pp. 361–373, 2014.

[22] X. Xu, W. Chen, R. Miao et al., "miR-34a induces cellular senescence via modulation of telomerase activity in human hepatocellular carcinoma by targeting FoxM1/c-Myc pathway," *Oncotarget*, vol. 6, no. 6, pp. 3988–4004, 2015.

[23] H. Tazawa, N. Tsuchiya, M. Izumiya, and H. Nakagama, "Tumor-suppressive miR-34a induces senescence-like growth arrest through modulation of the E2F pathway in human colon cancer cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 39, pp. 15472–15477, 2007.

[24] C. Welch, Y. Chen, and R. L. Stallings, "MicroRNA-34a functions as a potential tumor suppressor by inducing apoptosis in neuroblastoma cells," *Oncogene*, vol. 26, no. 34, pp. 5017–5022, 2007.

[25] V. P. Tryndyak, S. A. Ross, F. A. Beland, and I. P. Pogribny, "Down-regulation of the microRNAs miR-34a, miR-127, and miR-200b in rat liver during hepatocarcinogenesis induced by a methyl-deficient diet," *Molecular Carcinogenesis*, vol. 48, no. 6, pp. 479–487, 2009.

[26] T. Sukata, K. Sumida, M. Kushida et al., "Circulating microRNAs, possible indicators of progress of rat hepatocarcinogenesis from early stages," *Toxicology Letters*, vol. 200, no. 1-2, pp. 46–52, 2011.

[27] X. Y. Bai, Y. Ma, R. Ding, B. Fu, S. Shi, and X. M. Chen, "miR-335 and miR-34a promote renal senescence by suppressing mitochondrial antioxidative enzymes," *Journal of the American Society of Nephrology: JASN*, vol. 22, no. 7, pp. 1252–1261, 2011.

[28] K. Kumamoto, E. A. Spillare, K. Fujita et al., "Nutlin-3a activates p53 to both down-regulate inhibitor of growth 2 and up-regulate mir-34a, mir-34b, and mir-34c expression, and induce senescence," *Cancer Research*, vol. 68, no. 9, pp. 3193–3203, 2008.

[29] H. C. He, Z. D. Han, Q. S. Dai et al., "Global analysis of the differentially expressed miRNAs of prostate cancer in Chinese patients," *BMC Genomics*, vol. 14, p. 757, 2013.

[30] L. Chen, Y. Li, Y. Fu et al., "Role of deregulated microRNAs in breast cancer progression using FFPE tissue," *PLoS One*, vol. 8, no. 1, article e54213, 2013.

[31] N. M. White, H. W. Khella, J. Grigull et al., "miRNA profiling in metastatic renal cell carcinoma reveals a tumour-suppressor effect for miR-215," *British Journal of Cancer*, vol. 105, no. 11, pp. 1741–1749, 2011.

[32] K. Nakazawa, N. Dashzeveg, and K. Yoshida, "Tumor suppressor p53 induces miR-1915 processing to inhibit Bcl-2 in the apoptotic response to DNA damage," *The FEBS Journal*, vol. 281, no. 13, pp. 2937–2944, 2014.

[33] C. Xu, H. Li, L. Zhang, T. Jia, L. Duan, and C. Lu, "MicroRNA19153p prevents the apoptosis of lung cancer cells by downregulating DRG2 and PBX2," *Molecular Medicine Reports*, vol. 13, no. 1, pp. 505–512, 2016.

[34] N. Susnow, L. Zeng, D. Margineantu, and D. M. Hockenbery, "Bcl-2 family proteins as regulators of oxidative stress," *Seminars in Cancer Biology*, vol. 19, no. 1, pp. 42–49, 2009.

[35] D. Li, Q. Wang, C. Liu et al., "Aberrant expression of miR-638 contributes to benzo(a)pyrene-induced human cell transformation," *Toxicological Sciences: An Official Journal of the Society of Toxicology*, vol. 125, no. 2, pp. 382–391, 2012.

[36] Y. Tay, S. M. Tan, F. A. Karreth, J. Lieberman, and P. P. Pandolfi, "Characterization of dual PTEN and p53-targeting microRNAs identifies microRNA-638/Dnm2 as a two-hit oncogenic locus," *Cell Reports*, vol. 8, no. 3, pp. 714–722, 2014.

[37] D. S. Gridley, T. L. Freeman, A. Y. Makinde et al., "Comparison of proton and electron radiation effects on biological responses in liver, spleen and blood," *International Journal of Radiation Biology*, vol. 87, no. 12, pp. 1173–1181, 2011.

[38] X. X. Wang, J. Liu, Y. M. Tang, L. Hong, Z. Zeng, and G. H. Tan, "MicroRNA-638 inhibits cell proliferation by targeting suppress PIM1 expression in human osteosarcoma," *Tumour Biology: The Journal of the International Society for Oncodevelopmental Biology and Medicine*, vol. 37, no. 12, pp. 16367–16375, 2016.

[39] J. Zhang, Z. Bian, J. Zhou et al., "MicroRNA-638 inhibits cell proliferation by targeting phospholipase D1 in human gastric carcinoma," *Protein & Cell*, vol. 6, no. 9, pp. 680–688, 2015.

[40] J. Zhang, B. Fei, Q. Wang et al., "MicroRNA-638 inhibits cell proliferation, invasion and regulates cell cycle by targeting tetraspanin 1 in human colorectal carcinoma," *Oncotarget*, vol. 5, no. 23, pp. 12083–12096, 2014.

[41] A. Bhattacharya, U. Schmitz, Y. Raatz et al., "miR-638 promotes melanoma metastasis and protects melanoma cells from apoptosis and autophagy," *Oncotarget*, vol. 6, no. 5, pp. 2966–2980, 2015.

[42] Y. Ren, Y. Chen, X. Liang, Y. Lu, W. Pan, and M. Yang, "MiRNA-638 promotes autophagy and malignant phenotypes of cancer cells via directly suppressing DACT3," *Cancer Letters*, vol. 390, pp. 126–136, 2017.

[43] N. Wang, Z. Zhou, T. Wu et al., "TNF-alpha-induced NF-kappaB activation upregulates microRNA-150-3p and inhibits osteogenesis of mesenchymal stem cells by targeting beta-catenin," *Open Biology*, vol. 6, no. 3, 2016.

[44] M. P. Borgohain, M. Lahkar, S. Ahmed et al., "Small molecule inhibiting nuclear factor-kB ameliorates oxidative stress and suppresses renal inflammation in early stage of alloxan-induced diabetic nephropathy in rat," *Basic & Clinical Pharmacology & Toxicology*, vol. 120, no. 5, pp. 442–449, 2017.