RESEARCH ARTICLE

MicroRNA-146a-5p enhances radiosensitivity in hepatocellular carcinoma through replication protein A3-induced activation of the DNA repair pathway

Jing Luo,1 Zhong-Zhou Si,1 Ting Li,1 Jie-Qun Li,1 Zhong-Qiang Zhang,1 Guang-Shun Chen,1 Hai-Zhi Qi,1* and Hong-Liang Yao2*

1Department of Organ Transplantation and General Surgery, Second Xiangya Hospital of Central South University, Changsha, China; and 2Department of Gastrointestinal Surgery, Second Xiangya Hospital of Central South University, Changsha, China

Submitted 14 May 2018; accepted in final form 17 November 2018

Hepatocellular carcinoma (HCC) falls under the category of heterogeneous diseases, and its development is often caused by various etiologies, including hepatitis B virus (HBV), metabolic syndrome, and chronic alcohol abuse (25). At present, HCC is the fifth most frequently occurring type of cancer, which accounts for ~5% of all cancers worldwide, and its incidence is on the rise, with more than 500,000 new cases reported each year (10). The treatment for HCC patients depends on the different stages of the disease, which includes ablation, transplantation or resection at an early stage, chemoembolization at an intermediate stage, kinase inhibitor sorafenib at advanced stage, and transplantation at end stage (3). In the advanced stages of HCC, radiation therapy is considered to be an effective therapeutic method for tumor control, which includes internal radiation therapy with radioisotopes (15). Although radiotherapy is considered to be a major therapeutic option for HCC patients, its efficacy is under the limitation of intrinsic radio resistance of the tumor, which is influenced mainly by the activity of the DNA damage repair pathway (5). MicroRNAs (miRNAs) can efficiently attain tumor radiosensitivity control during the different stages of the disease through its effects on the radio-related signal transduction pathways, DNA damage repair, tumor microenvironment, apoptosis, and cell cycle checkpoint (36).

miRNAs are short noncoding RNAs, and miR-146a-5p has been found to be the most downregulated miRNA during liver fibrosis (8). In Wharton’s jelly mesenchymal stem cells (WJ-MSCs), a decrease in miR-146a-5p could inhibit the proliferation and enhance the migration of these stem cells (12). Meanwhile, the carcinogenesis and deterioration of HCC are influenced by the downregulation of miR-146a-5p expression, which indicates that the upregulation of miR-146a-5p could lead to HCC suppression (35). Replication protein A (RPA) is a single-stranded (ssDNA)-binding protein that can affect DNA recombination, DNA repair, and replication, and cell cycle checkpoint and RPA3 is its subunit (29). An increase in the expression of RPA3 plays an important role in nasopharyngeal carcinoma (NPC) radio resistance and is expected to play a role as a biomarker in predicting radiosensitivity and prognosis in NPC patients (24). In addition, the upregulation of RPA3 expression could promote tumor progression in HCC cells, which leads to high patient mortality (29). DNA repair pathways can be improperly activated by cancer cells to overcome standard anticancer treatments with preserved genome integrity (21). If repair is impossible, normal cells will promote DNA damage repair and cause cell cycle arrest and apoptosis through DNA repair pathways (2). In this study, RPA3 was identified as the target gene of miR-146a-5p based on the microRNA.org biology prediction website. In the present...
study, we aimed at investigating the radiosensitivity of HCC, with RPA3 as the target gene of miR-146a-5p, and the influence of miR-146a-5p on DNA repair pathways.

MATERIALS AND METHODS

Ethics statement. The study was approved by the Institutional Review Board of the Second Xiangya Hospital of Central South University. The experiments were conducted in accordance with the declaration of Helsinki. Written informed consent forms were obtained from each participating patient or family member.

Bioinformatics prediction. Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/) was used to retrieve the data of HCC chip to obtain the GSE40367 expression chip. In HCC without metastasis and primary tumors, the limma package of R language was used.

| Table 1. Primer sequences of miR-146a-5p, U6, ATM, pCHK2, Rad51, and GAPDH used for RT-qPCR |
|-----------------------------------------------------------------------------------------------|
| **Target Gene** | **Forward Primer (5′–3′)** | **Reverse Primer (5′–3′)** |
|------------------|-----------------------------|-----------------------------|
| miR-146a-5p      | ACGCTTGTCGTGCTTACGGAT       | CCATGCTGTCAAGGAGAAGAC       |
| U6               | GAGGGGCTTATTTCCCATGATT      | TAATTAGAATTATTTTGACT        |
| RPA3             | ATCGAGGCTCAAAGAAGAGCTTC     | AGTTGAGGCACCTTTTCTTTCTT     |
| ATM              | TTTAGGCCACTCTGCTGGG          | TGGCGTAGAAGGGCAGCTGAT       |
| pCHK2            | AGCGAGCCTCTAGACGAAGA         | CGAGAGCAGCAAGAAGGTT         |
| Rad51            | CAGCCGTGTTTTACAGTTTAGAG     | CAATGAGGAGCTGGCCTAGT        |
| GAPDH            | ACGGCTGTGTTTAAACTCTGCTG     | CCCGACCTTAGTTGTGGAGGA        |

ATM, ataxia-telangiectasia mutated; pCHK2, phosphorylation of checkpoint kinase 2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; miR-146a-5p, microRNA-146a-5p; RT-qPCR, reverse transcription quantitative polymerase chain reaction; RPA3, replication protein A3.

Fig. 1. MicroRNA-146a (miR-146a) contributes to the improved effect of radiotherapy on hepatocellular carcinoma (HCC) through binding to replication protein A3 (RPA3) and the DNA repair pathway. A: heat map of differentially expressed genes in HCC expression chip. The x-axis represents sample number and the y-axis represents genes. Left: dendrogram represents gene expression cluster in which each cube represents the level of a gene in a sample. Right: histogram is color gradation, which represents gene level and sample type; red shows high gene level, whereas green exhibits low gene level. B–D: levels of the most significant three genes in the The Cancer Genome Atlas (TCGA) of the HCC database; x-axis represents sample type and y-axis represents expression level. Left: blue box plot represents gene level in the normal sample; right: red box plot represents gene levels in HCC sample. E: prediction on regulative miRNA of RPA3; blue represents prediction results of the DIANA database, red represents prediction results of mirDIP database, and green represents prediction results of the TargetScan database; the middle part represents the intersection of prediction results. *P < 0.05 vs. normal group.
used for the differential analysis of HCC with lung metastasis and metastasis tumor samples. logFCi > 2 and P < 0.05 were set as the threshold to screen out differentially expressed genes. Next, UALCAN (http://ualcan.path.uab.edu/index.html), a user-friendly, interactive web resource for analyzing cancer transcriptionome data, was used to retrieve the expression of acyl-CoA thioesterase 12 (ACOT12), RPA3 and STAT4 in The Cancer Genome Atlas (TCGA) of HCC expression data. Next, DIANA database (http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=miR_CDS/index), mirDIP database (http://ophid.utoronto.ca/mirDIP/index.jsp#r), and TargetScan database (http://www.targetscan.org/vert_71/) were used to determine the regular miRNAs of RPA3. A Venn diagram was constructed via the website http://bioinformatics.psb.ugent.be/webtools/Venn/ to determine the intersection values of the predicted results of the three databases.

**Subject selection.** Liver tissues were resected from 69 patients diagnosed with HCC through laparoscopic surgery from October 2011 to August 2015 at the Second Xiangya Hospital of Central South University. Of the 69 patients, 61 were men and 8 were women. The patients’ ages ranged from 36–74 yr, with an average age of 53.7 yr. The follow-up period was between 3.3 and 5.8 yr. The Union for International Cancer Control on cancer tumor-nodes-metastasis classification method was used for tumor staging and grading (22). The inclusion criteria included 1) patients with a complete history and clinical data, 2) patients whose HCC diagnosis had been confirmed through pathological findings, 3) patients who had not received any chemotherapy, immune therapy, surgical treatment, or radiotherapy before the operation, 4) patients who were in a good condition on the basis of an evaluation and Karnofsky Performance score > 70 (1), and 5) patients with normal hepatic and renal function and regular electrocardiograms and who did not have other chemotherapy contraindications (leucocyte counts > 4.0 x 10^9/L, platelet counts > 100 x 10^9/L, hemoglobin > 120 g/L). The exclusion criteria were as follows: 1) patients who had received radiosensitive and radioprotective agent treatment apart from radiotherapy and chemotherapy, 2); patients who had previously received surgical treatments or some form of chemoradiotherapy, and 3) patients with previous history of tumors.

**Radiotherapy regimen and efficacy determination.** Patients were asked to lie in a supine position on the treatment table. Enhanced CT scanning was performed on the patients with an interval of 3 mm. The two ends of the lesion were scanned by continuous CT with an interval of 5 mm. The imaging data were input into the UNICORN 3DTPS treatment planning system through the network, and the organs at risk were delineated by a physician. The radiation dose used on patients ranged from 250 to 300 cGy every day. The radiation field was narrowed, and 10–20 Gy was added depending on the decrease in tumor size. The 6 Mv-X-ray produced by the linear accelerator (Varian 600; Varian Medical Systems, Palo Alto, CA) was used to radiate patients with a dose rate of 200 cGy/minute. The tumor tissues were extracted following radiotherapy.

According to the Response Evaluation Criteria in Solid Tumors (RECISt1.1), the therapeutic evaluation (28) was divided into complete remission (CR), partial remission (PR), stable condition (SC), and disease progression (PD). Recent radiotherapy sensitivity = CR + PR; recent radiotherapy insensitivity = SC + PD.

**Cell culture.** HCC cells [SMMC-7721 (female), HepG2 (male), Hep-3B (male), QGY-7703 (female), and Bel-7402 (female); Shanghai Ai Yansheng Biotechnology, Shanghai, China] were tested by the short tandem repeat (STR) typing method and cultured in 5 ml of Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum (FBS). After several subcultures, the follow-up experiments were carried out once the cells reached a desirable confluency.

**Immunohistochemistry.** Small cubes of HCC tissues and paracancerous tissues were fixed by 10% formaldehyde solution for 24 h. Paraffin-embedded tissues were cut into 4-μm serial sections and then incubated at 60°C for 1 h. Tissue slices were then dewaxed with conventional xylene, dehydrated with gradient ethanol, and placed in 3% H2O2 for 10 min. Following high antigen pressure repair for 1–3 min, 10% normal goat serum blocking solution (Beijing Kangwei Medical Systems, Palo Alto, CA) was used to radiate patients with a dose rate of 200 cGy/minute. The tumor tissues were extracted following radiotherapy.

**Radiotherapy regimen and efficacy determination.** Patients were asked to lie in a supine position on the treatment table. Enhanced CT scanning was performed on the patients with an interval of 3 mm. The two ends of the lesion were scanned by continuous CT with an interval of 5 mm. The imaging data were input into the UNICORN 3DTPS treatment planning system through the network, and the organs at risk were delineated by a physician. The radiation dose used on patient was not higher than the respective tolerance dose. The apy and chemotherapy, and different sizes of radiation fields were adopted according to different pathological properties. The whole liver was divided into several longitudinal segments with a width of 2.5 cm and was treated by 6 Mv-X-ray radiations. Radiation therapy started from the first segment on the right side, and the ventral surface was radiated in the same way as the dorsal surface. The radiation used on patients ranged from 250 to 300 cGy every day. The radiation field was narrowed, and 10–20 Gy was added depending on the decrease in tumor size. The 6 Mv-X-ray produced by the linear accelerator (Varian 600; Varian Medical Systems, Palo Alto, CA) was used to radiate patients with a dose rate of 200 cGy/minute. The tumor tissues were extracted following radiotherapy.

According to the Response Evaluation Criteria in Solid Tumors (RECISt1.1), the therapeutic evaluation (28) was divided into complete remission (CR), partial remission (PR), stable condition (SC), and disease progression (PD). Recent radiotherapy sensitivity = CR + PR; recent radiotherapy insensitivity = SC + PD.

**Cell culture.** HCC cells [SMMC-7721 (female), HepG2 (male), Hep-3B (male), QGY-7703 (female), and Bel-7402 (female); Shanghai Ai Yansheng Biotechnology, Shanghai, China] were tested by the short tandem repeat (STR) typing method and cultured in 5 ml of Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum (FBS). After several subcultures, the follow-up experiments were carried out once the cells reached a desirable confluency.

**Immunohistochemistry.** Small cubes of HCC tissues and paracancerous tissues were fixed by 10% formaldehyde solution for 24 h. Paraffin-embedded tissues were cut into 4-μm serial sections and then incubated at 60°C for 1 h. Tissue slices were then dewaxed with conventional xylene, dehydrated with gradient ethanol, and placed in 3% H2O2 for 10 min. Following high antigen pressure repair for 1–3 min, 10% normal goat serum blocking solution (Beijing Kangwei Medical Systems, Palo Alto, CA) was used to radiate patients with a dose rate of 200 cGy/minute. The tumor tissues were extracted following radiotherapy.

According to the Response Evaluation Criteria in Solid Tumors (RECISt1.1), the therapeutic evaluation (28) was divided into complete remission (CR), partial remission (PR), stable condition (SC), and disease progression (PD). Recent radiotherapy sensitivity = CR + PR; recent radiotherapy insensitivity = SC + PD.

**Cell culture.** HCC cells [SMMC-7721 (female), HepG2 (male), Hep-3B (male), QGY-7703 (female), and Bel-7402 (female); Shanghai Ai Yansheng Biotechnology, Shanghai, China] were tested by the short tandem repeat (STR) typing method and cultured in 5 ml of Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum (FBS). After several subcultures, the follow-up experiments were carried out once the cells reached a desirable confluency.

**Immunohistochemistry.** Small cubes of HCC tissues and paracancerous tissues were fixed by 10% formaldehyde solution for 24 h. Paraffin-embedded tissues were cut into 4-μm serial sections and then incubated at 60°C for 1 h. Tissue slices were then dewaxed with conventional xylene, dehydrated with gradient ethanol, and placed in 3% H2O2 for 10 min. Following high antigen pressure repair for 1–3 min, 10% normal goat serum blocking solution (Beijing Kangwei Century Biotechnology, Beijing, China) was added to the slices. The slices were then incubated overnight at 4°C with appropriate rabbit anti-human monoclonal antibodies ATM (AB_1640207, 1: 50,000, ab811292; Abcam), pCHK2 (AB_10863751, 1: 200,000, ab109413, Abcam), and Rad51 (AB_2722613, 1: 10,000; ab135354; Abcam). Next, the slices were added with biotin-labeled secondary antibody and incubated at 37°C for 30 min, followed by treatment with streptavidin-peroxidase solution (Beijing Zhongshan Biotechnology, Beijing, China). Following staining with diaminobenzidine for 5–10 min, the slices were washed with distilled water for 10 min, immersed in hematoxylin for 4 min, washed with running water, and rinsed with ethanol for 10 s. After bathing in running water, ammonia was added to obtain a blue color. The slices were dehydrated, cleared, and mounted with neutral balsam. The positively stained area and its percentage in the total area were measured in five randomly selected high-power fields.

**Fig. 2.** Replication protein A3 (RPA3) is upregulated, whereas microRNA (miR)-146a-5p and acyl-CoA thioesterase 12 (ACOT12) are downregulated in hepatocellular carcinoma (HCC) tissues. A: the miR-146a-5p expression and mRNA expression of RPA3 and ACOT12 in HCC tissues and paracancerous tissues determined by RT-quantitative PCR. B and C: protein bands and statistical analysis of RPA3 and ACOT12 in HCC tissues and paracancerous tissues measured by Western blot analysis. Results are measurement data expressed as means ± SD and analyzed by unpaired t-test; n = 69, *P < 0.05 vs. paracancerous tissues.
miR-146a-5p TARGETING RPA3 IN HCC

Radiation treatment. HepG2, Hep-3B, and SMMC-7721 cells and the QGY-7703 and Bel-7402 cell lines were cultured separately. Afterward, the cells in each medium were separately irradiated by different radioactive rays (0, 2, 4, 6, and 8) Gy from 6Mv-X-ray produced by a linear accelerator (Varian 600; Varian Medical Systems) simultaneously. The following day after irradiation, HCC cells in each medium were detected by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay to determine cell proliferation status. HCC cells with high radiosensitivity were selected for further experiments to determine the best radiation treatment plan, and then the transfected cells in different groups were treated with the most appropriate and effective radiation plan.

Dual-luciferase reporter assay. The online biological prediction website microRNA.org was used for the analysis of target genes of miR-146a-5p. The length of 3′-untranslated region (3′-UTR) area of RPA3 gene was expanded by clone (forward: 5′-CATCATCACCATCACCTGCT-3′; reverse: 5′-AGGGTAAGCCATTGCATCTG-3′). The plasmid extracted was then connected with luciferase report carrier pmirGLO (E1330; Promega, Madison, WI) by target segment synthesized by Shanghai Yihe Applied Biotechnology (Shanghai, China) to obtain cDNA. Primers and probes of miR-146a-5p, RPA3, ATM, pCHK2, and Rad51 were designed and synthesized by Shanghai Yibre Applied Biotechnology (Shanghai, China) (Table 1). Quantitative PCR ABI7500 (ABI, Oyster Bay, NY) was applied in RT-quantitative (q)PCR experiment. U6 was considered as the internal control of miR-146a-5p, whereas glyceraldehyde-

Fig. 3. Hepatocellular carcinoma (HCC) tissues have higher expression rates of ataxia-telangiectasia mutated (ATM), phosphorylation of checkpoint kinase 2 (pCHK2), and Rad51. A: immunohistochemical staining map of HCC tissues and paracancerous tissues (ATM, pCHK2, and Rad51) ×200. ATM, pCHK2, and Rad51 were expressed in cytoplasm and nuclei. B: quantitative analysis indicates that expression of ATM, pCHK2, and Rad51 was upregulated in HCC tissues. Results are measurement data expressed as means ± SD and analyzed by unpaired t-test; n = 69, *P < 0.05 vs. paracancerous tissues.

Fig. 4. Hepatocellular carcinoma (HCC) tissues have higher microRNA (miR)-146a-5p but lower Replication protein A3 (RPA3) mRNA expression. Results are measurement data expressed as means ± SD and analyzed by unpaired t-test; n = 69, *P < 0.05 vs. preradiation.
3-phosphate dehydrogenase (GAPDH) served as the internal reference of RPA3, ATM, and pCHK2. The reliability of the PCR results was evaluated using the solubility curve. Relative expression of the target gene was calculated using the equation $2^{-\Delta\Delta C_{T}}$.

Western blot analysis. The radio-immunoprecipitation assay (RIPA) buffer (3 ml/g) was added into transfected SMMC-7721 and HepG2 cells. The cells were then lysed to obtain the protein samples of each group, and protein concentration was determined afterward. Proteins were separated by electrophoresis and subsequently transferred onto a nitrocellulose membrane and then blocked by 5% skim milk powder overnight at 4°C. Following the addition of diluted primary antibodies rabbit-anti-human monoclonal antibody RPA3 (AB_10860648, 1: 2,000, ab109394; Abcam), ACOT12 (AB_10860648, 1: 2,000, ab83326; Abcam), ATM (AB_1640207, 1: 5,000, ab109394; Abcam), Rad51 (AB_2722613, 1: 1,000, ab133534; Abcam), and rabbit polyclonal antibody GAPDH (AB_307275, 1: 2,500, ab9485; Abcam), the mixture was incubated overnight. The secondary antibody was diluted 103). Each type of cell was inoculated into three wells with 100/well in the 0 Gy group, 200/well in the 2 Gy group, 400/well in the 4 Gy group, 800/well in the 6 Gy group, 1,000/well in the 8 Gy group, and 2,000/well in 10 Gy group, respectively. Following the addition of cell suspension and 2 ml of culture medium in each well of a six-well plate, cells were further cultured at 37°C with 5% CO2 for 14 days. After clone formation in the culture plate, the culture was terminated, followed by the addition of crystal violet staining for 30 min. After crystal violet was washed out, the plate was dried with air. The plate was then reversed, and clones with more than 50 cells were counted at last = cloning efficiency of irradiation cell/cloning efficiency of the control cell.

MTT assay. When the growth density of transfected cells reached ~80%, transfected SMMC-7721 and HepG2 cells in each group were seeded in a 96-well plate at a density of 5 × 10^7 cells/l, followed by the addition of 20 μl of MTT solution (5 g/l) into each well after being cultured for 24, 48, and 72 h. The supernatant culture medium was discarded after 4 h, and 150 μl of dimethyl sulfoxide (DMSO) was added into each well and gently shaken for 10 min. The optical density

**Fig. 5.** microRNA (miR)-146a-5p is highly expressed and replication protein A3 (RPA3) is poorly expressed in responders. A: miR-146a-5p expression before and after radiotherapy in hepatocellular carcinoma (HCC) tissues. B: RPA3 mRNA expression before and after radiotherapy in HCC tissues. Results are measurement data expressed as means ± SD and analyzed by unpaired t-test. Responders, n = 52; nonresponders, n = 17. *P < 0.05 vs. preradiation.

**Fig. 6.** Replication protein A3 (RPA3) targets microRNA (miR)-146a-5p. A: binding regions between RPA3 3′-untranslated region (3′-UTR) and miR-146a-5p sequence. B: luciferase activity of the RPA3 wild type (WT) and RPA3 mutant type (MUT) in human embryonic kidney-293T cells after transfection. The experiment was repeated 3 times. Results are measurement data expressed as means ± SD and analyzed by unpaired t-test. *P < 0.05 vs. negative control (NC) group.
detached using 0.25% trypsin. The adjusted sample cell number was cell cycle. After transfection for 48 h, the cells were collected and 5 binding buffer and uniformly mixed with 10/H9262, and 5 following centrifugation, the cells were resuspended in 200 cml of cell suspension at 4°C. PI staining solution (1 ml, 50 mg/l) containing RNAase was added into 100 μl of cell suspension (10^6 cells/ml). The cells were kept with the avoidance of light for 30 min and filtered with nylon net with 300 meshes. The samples were analyzed using a flow cytometer to detect cell cycle by red fluorescence at the excitation wavelength of 488 nm.

Cell apoptosis was detected by Annexin V-FITC/PI staining. Cell treatment was the same with cell cycle analysis. Cells were cultured at 37°C and 5% CO2 for 48 h, collected, and washed with PBS twice. Following centrifugation, the cells were resuspended in 200 μl of binding buffer and uniformly mixed with 10 μl of Annexin V-FITC and 5 μl of PI, and a reaction took place with the avoidance of light at room temperature for 15 min before adding 300 μl of binding buffer. Cell apoptosis was detected by flow cytometry (6HT, Wuhan Cellwar Bio-technology, Wuhan, Hubei, China) at the excitation wavelength of 488 nm.

Statistical analysis. All data were analyzed by SPSS 21.0 (IBM, Armonk, NY). Measurement data were expressed as means ± SD. First, the normality test and variance homogeneity test were carried out. Unpaired t-test (independent-samples t-test) was performed to test data with normal distribution and homogeneous variance, whereas data with normal distribution and inhomogeneous variance were determined with Welch’s t-test. The repeated-measures analysis of variance was employed for cell proliferation. Comparisons among multiple groups were performed with one-way analysis of variance with the Tukey’s post hoc tests for multiple pairwise comparisons. If the data did not conform to the normality of data or the homogeneity of variance, the rank-sum test was used. When P < 0.05, the difference was considered statistically significant.

RESULTS

MiR-146a improves the effect of radiotherapy on HCC by binding to RPA3 and the DNA repair pathway. HCC expression chip GSE40367 was obtained, and only 26 differentially expressed genes were obtained by differential expression analysis of the sample. The heat map (Fig. 1A) was constructed on 26 differentially expressed genes. The results showed that compared with the control group, 18 genes had markedly elevated levels and eight genes presented with decreased levels. STAT4, ACOT12, and RPA3 were the three genes with the most significant difference between the minimum P value. Levels of these three genes in TCGA of the HCC samples and normal sample were further retrieved (Fig. 1, B–D), and the findings revealed significantly increased levels in RAP3 and decreased levels in ACOT12, whereas there were no significant changes observed in STAT4 levels. Based on further information retrieval on the study of RPA3 and ACOT12 on HCC, there were no related reports that found the effect of ACOT12 on HCC, whereas there were a few reports regarding RPA3 on HCC (26, 29). In addition, among the study on RPA3, researchers pointed out that RPA3 showed prominently upregulated levels in HCC and promoted cell proliferation and invasion of HCC. These results were in line with what we have obtained through chip analysis and TCGA expression data analysis. Although the related function of RPA3 on HCC has been reported, there is very little known regarding the effect of RPA3 on radiotherapy of HCC. Further information retrieved on RPA3-related signaling pathway has demonstrated that RPA3 is closely associated with the DNA repair pathway (11, 24), and DNA repair has been confirmed to play a role in tumor radiotherapy (9, 23). The above analysis and related reports revealed that RPA3 may affect radiotherapy of HCC through the DNA repair pathway. To get more information about the

Fig. 7. The proliferation of SMMC-7721 and HepG2 cells is markedly inhibited after radiation with 8-Gy, 6-Mv doses. The proliferation rate of SMMC-7721 and HepG2 cells decreased the most after irradiation of the 8-Gy dose. The experiment was repeated 3 times. The experiment subjects were SMMC-7721, HepG2, Hep-3B, QGY-7703, and Bel-7402 cells. Results are measurement data expressed as means ± SD and analyzed by the repeated measurement analysis of variance. *P < 0.05 vs. SMMC-7721 and HepG2 cells. OD, optical density.

Fig. 8. MicroRNA (miR)-146a-5p activates the DNA damage repair pathway via downregulation of replication protein A3 (RPA3). A: microRNA (miR)-146a-5p expression and mRNA expression of RPA3, ataxia-telangiectasia mutated (ATM), phosphorylation of checkpoint kinase 2 (pCHK2), and Rad51 in SMMC-7721 cells. B: miR-146a-5p expression and mRNA expression of RPA3, ATM, pCHK2, and Rad51 in HepG2 cells. Results are measurement data expressed as means ± SD and analyzed by 1-way analysis of variance. The experiment subjects are SMMC-7721 and HepG2 cells; the experiment was repeated 3 times. *P < 0.05 vs. blank and negative control (NC) groups; #P < 0.05 vs. X-ray group.
function mechanism of RPA3 on HCC, databases like DIANA were used to predict regulative miRNA of RPA3. The findings signified 29 regulative miRNAs in the DIANA database, and seven regulative miRNAs were predicted in the mirDIP database, whereas 5 miRNAs of conserved sites were predicted in the TargetScan database. To improve the accuracy, the top 15 miRNAs in the DIANA database and prediction results of mirDIP and TargetScan databases were analyzed through Venn histogram (Fig. 1E). The results showed that only two miRNAs, miR-146a and miR-146b, were located in the prediction result intersection of three databases. The further information retrieval about the study of these 2 miRNAs on HCC showed that a few reports have revealed that miR-146a is closely related to HCC progression (18, 34, 37). These findings dem-

Fig. 9. MicroRNA (miR)-146a-5p inhibits replication protein A3 (RPA3) protein expression but promotes ataxia-telangiectasia mutated (ATM), phosphorylation of checkpoint kinase 2 (pCHK2), and Rad51 protein expression. A: value of RPA3, ATM, pCHK2, and Rad51 protein bands in SMMC-7721 cells (gray). B: protein levels of RPA3, ATM, pCHK2, and Rad51 in SMMC-7721 cells. C: value of RPA3, ATM, pCHK2, and Rad51 protein bands in HepG2 cells (gray). D: protein levels of RPA3, ATM, pCHK2, and Rad51 in HepG2 cells. The experiment subjects were SMMC-7721 and HepG2 cells; the experiment was repeated 3 times. Results are measurement data expressed as means ± SD and analyzed by one-way analysis of variance. *P < 0.05 vs. blank and negative control (NC) groups; #P < 0.05 vs. X-ray group.
miR-146a-5p TARGETING RPA3 IN HCC

A microRNA (miR)-146a-5p overexpression improves radiosensitivity of hepatocellular carcinoma (HCC) cells. A: radiosensitivity of SMMC-7721 cells in response to the treatment of miR-146a-5p mimic, miR-146a-5p inhibitor, X-ray, miR-146a-5p mimic + X-ray, and miR-146a-5p inhibitor + X-ray detected by clonogenic assay. B: radiosensitivity of HepG2 cells in response to the treatment of miR-146a-5p mimic, miR-146a-5p inhibitor, X-ray, miR-146a-5p mimic + X-ray, and miR-146a-5p inhibitor + X-ray detected by clonogenic assay. Results are measurement data expressed as means ± SD and analyzed by the repeated-measures analysis of variance.

miR-146a-5p is upregulated whereas RPA3 is downregulated in postradiotherapy effective cases. Following radiotherapy, the expression of miR-146a-5p and RPA3 in HCC tissues was further assessed. Among the 69 patients with HCC treated with an 8-Gy, 6-Mv dose of radiation, 52 cases presented with radiotherapy sensitivity, with one case of CR and 52 cases of PR, and 17 cases were found with radiotherapy insensitivity, with 15 cases of SC and two cases of PD. Compared with preradiation, the radiotherapy sensitivity group demonstrated increased expression of miR-146a-5p (P < 0.05), whereas there was a decline in mRNA expression of RPA3 (P < 0.05). In the radiotherapy insensitivity group, the expression of miR-146a-5p showed no significant difference (P > 0.05), whereas there was an only slight enhancement in mRNA expression of RPA3 (P > 0.05) (Fig. 5, A and B).

**RPA3 is a target gene of miR-146a-5p.** According to the biology prediction website available at microRNA.org, RPA3 was identified as the target gene of miR-146a-5p (Fig. 6A). Dual-luciferase reporter assay was conducted to confirm this prediction. The results showed that, compared with the NC group, luciferase activity of the RPA3-WT 3′-UTR was significantly inhibited by miR-146a-5p (P < 0.05), whereas there was no inhibition observed in terms of luciferase activity of the RPA3 MUT 3′-UTR (Fig. 6B). These findings suggested that miR-146a-5p was bound specifically to the RPA3 3′-UTR and reduced RPA3 gene expression following transcription.

**HCC tissues have higher positive expression rate of ATM, pCHK2, and Rad51.** After immunohistochemistry was performed to test the positive expression rate of ATM, pCHK2, and Rad51, the results showed that ATM, pCHK2, and Rad51 were expressed in cytoplasm and nuclei, and the positive rate of ATM, pCHK2, and Rad51 in HCC tissues was higher than in paracancerous tissues (P < 0.05; Fig. 3, A and B).

**HCC tissues have higher miR-146a-5p but lower RPA3 mRNA expression.** The miR-146a-5p and RPA3 expression in HCC tissues was detected, and the results showed that 69 HCC patients treated with the same dose (8 Gy and 6 Mv) of radioactive rays had significantly increased miR-146a-5p but markedly decreased RPA3 mRNA expression in HCC tissues when compared with preradiation (P < 0.05; Fig. 4).

**MiR-146a-5p affects radiation sensitivity in HCC cells.** The experiment subjects were SMMC-7721 and HepG2 cells; the experiment was repeated 3 times. Results are measurement data expressed as means ± SD and analyzed by the repeated-measures analysis of variance. The experiment subjects were SMMC-7721 and HepG2 cells; the experiment was repeated 3 times. *P < 0.05 vs. blank and negative control (NC) groups; #P < 0.05 vs. X-ray group. RPA3, replication protein A3.
Proliferation of SMMC-7721 and HepG2 cells is markedly inhibited following radiation with 8-Gy, 6-Mv doses. The effect of radiotherapy on proliferation of SMMC-7721, HepG2, Hep-3B, QGY-7703, and Bel-7402 cells was analyzed by MTT assay, and the results indicated that cell proliferation rates of SMMC-7721, HepG2, Hep-3B, QGY-7703, and Bel-7402 were reduced as the dose was increased ($P < 0.05$). SMMC-7721 and HepG2 presented with the highest radiosensitivity based on the falling range, which had the highest ranking ($P < 0.05$). The decrease in cell proliferation rate with the 8-Gy radiation dose was faster in SMMC-7721 and HepG2 (Fig. 7). These results indicated that proliferation of SMMC-7721 and HepG2 cells was significantly inhibited with the 8-Gy and 6-Mv radiation dose, and as a result they were selected for subsequent experiments.

**miR-146a-5p activates the DNA damage repair pathway by downregulation of RPA3.** The results from RT-qPCR and Western blot analysis that were conducted to determine the expression of RPA3 and the DNA damage repair pathway-related factors (ATM, pCHK2, and Rad51) showed that SMMC-7721 and HepG2 cells had the same trend (Figs. 8 and 9); the mRNA and protein expression of RPA3, ATM, pCHK2, and Rad51 showed no significant difference between the blank group and the NC group ($P > 0.05$). Compared with the blank group and the NC group, the miR-146a-5p mimic group and the miR-146a-5p inhibitor + X-ray group had significantly elevated expression of miR-146a-5p, and the miR-146a-5p mimic, X-ray, and miR-146a-5p mimic + X-ray groups demonstrated significantly increased mRNA and protein expression of ATM, pCHK2, and Rad51, whereas mRNA and protein expression of RPA3 was markedly inhibited (all $P < 0.05$); in the miR-146a-5p inhibitor group, the expression of miR-146a-5p and mRNA and protein expression of RPA3 was increased ($P < 0.05$); in the miR-146a-5p inhibitor + X-ray group, the expression of miR-146a-5p was

![Graphs showing the results of cell cycle analysis](image-url)

**Fig. 12.** Overexpressed microRNA (miR)-146a-5p inhibits cell cycle progression. **A**: flow cytometry cycle graph of SMMC-7721 cells. **B**: cell cycle percentage of SMMC-7721 cells in response to the treatment of miR-146a-5p mimic, miR-146a-5p inhibitor, X-ray, miR-146a-5p mimic + X-ray, and miR-146a-5p inhibitor + X-ray. The experiment subjects were SMMC-7721 and HepG2 cells; the experiment was repeated 3 times. Results are measurement data expressed as means ± SD and analyzed by repeated-measures analysis of variance. *$P < 0.05$ vs. blank and negative control (NC) groups; # $P < 0.05$ vs. the X-ray group.
lower, whereas mRNA and protein expression of other genes showed no significant change ($P > 0.05$). Compared with the X-ray group, the miR-146a-5p mimic + X-ray group illustrated significantly increased miR-146a-5p expression and mRNA and protein expression of ATM, pCHK2, and Rad51 and significantly decreased mRNA and protein expression of RPA3, whereas the miR-146a-5p inhibitor + X-ray group showed the opposite trends (all $P < 0.05$).

*Overexpressed miR-146a-5p improves radiosensitivity of HCC cells.* In the following experiments, radiosensitivity, proliferation, cycle, and apoptosis of HCC cells were determined, and the results from the clonogenic assay showed that

---

**Fig. 13.** Overexpressed microRNA (miR)-146a-5p promotes the apoptosis of hepatocellular carcinoma (HCC) cells. *A:* flow cytometry apoptosis graph of SMMC-7721 cells. *B:* comparison of flow cytometry apoptosis rate in SMMC-7721 cells in response to the treatment of miR-146a-5p mimic, miR-146a-5p inhibitor, X-ray, miR-146a-5p mimic + X-ray, and miR-146a-5p inhibitor + X-ray. *C:* flow cytometry apoptosis graph of HepG2 cells in each group. *D:* comparison of flow cytometry apoptosis rate in HepG2 cells in response to the treatment of miR-146a-5p mimic, miR-146a-5p inhibitor, X-ray, miR-146a-5p mimic + X-ray, and miR-146a-5p inhibitor + X-ray. The experiment subjects were SMMC-7721 and HepG2 cells; the experiment was repeated 3 times. Results are measurement data expressed as means ± SD and analyzed by one-way analysis of variance. *$P < 0.05$ vs. the blank and negative control (NC) groups; # $P < 0.05$ vs. the X-ray group.
SMCC-7721 and HepG2 cells had the same trend. No remarkable difference was found in the blank, NC, or miR-146a-5p inhibitor + X-ray groups (P > 0.05). Compared with the blank group and the NC group, the miR-146a-5p mimic, X-ray, and miR-146a-5p mimic + X-ray groups had decreased survival fraction of HCC cells and increased sensitivity (all P < 0.05). The survival fraction of HCC cells in the miR-146a-5p inhibitor group was increased, whereas sensitivity was decreased (P < 0.05; Fig. 10). Compared with the X-ray group, the miR-146a-5p mimic + X-ray group showed decreased cell survival fraction and increased sensitivity, whereas the miR-146a-5p inhibitor + X-ray group had increased cell survival fraction and decreased sensitivity (all P < 0.05).

Overexpression of miR-146a-5p reduces the proliferation of HCC cells. MTT assay was introduced to detect the proliferation of HCC cells. Based on the results, SMCC-7721 and HepG2 cells presented the same trend. There was no significant difference observed among the blank, NC, and miR-146a-5p inhibitor + X-ray groups (P > 0.05). Compared with the blank group and the NC group, the miR-146a-5p mimic, X-ray, and miR-146a-5p mimic + X-ray groups had decreased proliferation rate of HCC cells (all P < 0.05). Proliferation rate of HCC cells in the miR-146a-5p inhibitor group was increased (P < 0.05; Fig. 11). In comparison with the X-ray group, the miR-146a-5p mimic + X-ray group displayed decreased proliferation rate, whereas the miR-146a-5p inhibitor + X-ray group had an increased proliferation rate (all P < 0.05).

Overexpressed miR-146a-5p inhibits cell cycle progression and promotes the apoptosis of HCC cells. The cell apoptosis and cell cycle were determined. The results (Figs. 12 and 13) showed that SMCC-7721 and HepG2 cells had the same trend. There was no significant difference observed among the blank, NC, and miR-146a-5p inhibitor + X-ray groups (P > 0.05). Compared with the blank group and the NC group, the miR-146a-5p mimic, X-ray, and miR-146a-5p mimic + X-ray groups had a prolonged G0/G1 phrase and shortened S phase with higher apoptotic rate (P < 0.05) in HCC cells, and all index changes in the miR-146a-5p mimic + X-ray group were more obvious (all P < 0.05), whereas the miR-146a-5p inhibitor group showed shortened G0/G1 phase and prolonged S phase with lower apoptotic rate (P < 0.05). Compared with the X-ray group, the miR-146a-5p mimic + X-ray group demonstrated prolonged G0/G1 phase and shortened S phase with higher apoptotic rate (P < 0.05); the miR-146a-5p inhibitor + X-ray group displayed shortened G0/G1 phase and prolonged S phase with lower apoptotic rate (P < 0.05).

DISCUSSION

Despite the progress made in the treatment and the prognosis of HCC, it remains to be one of the most commonly occurring and major causes of mortality worldwide (16). The previous studies have demonstrated that miR-26b contributes to the enhancement of radiosensitivity by binding to erythropoietin, which produces human hepatocellular A2 in HCC (14). Furthermore, a recent study has illustrated that miRNA regulation can affect tumor radiosensitivity through various perspectives, including cell apoptosis and the DNA damage repair, making miRNA regulation an important factor that could be beneficial in tumor diagnosis and treatment, such as radiotherapy (36). In the present study, we aim to examine the regulatory mecha-
nism by which the biological function of miR-146a-5p plays a role in the enhancement of radiosensitivity in HCC, which in turn helps improve HCC treatment.

Initially, based on the target prediction program and the dual-luciferase activity determination, we found that RPA3 was a target gene of miR-146a-5p. The results from our study found that there was a decline in the expression of RPA3, whereas the expression of miR-146a-5p was elevated in HCC tissues and cell proliferation was restrained following radiotherapy. A previous study has demonstrated the effects of

![Map of molecular mechanisms involved in microRNA (miR)-146a-5p binding to replication protein A3 regulation (RPA3) in hepatocellular carcinoma radiosensitivity via the DNA damage repair pathway. miR-146a-5p was downregulated with weakened inhibitory effect, resulting in upregulated RPA3 expression and impaired DNA repair pathway as well as decreased apoptosis and increased hepatocellular carcinoma cell proliferation. With radiation treatment, miR-146a-5p was upregulated, RPA3 expression was inhibited, radiosensitivity and apoptosis were elevated, and proliferation was suppressed via activation of the DNA repair pathway.](C309)
miR-146a-5p downregulation in HCC and its potential to suppress tumors (35). Another study has demonstrated that the expression of RPA3 was upregulated in HCC cells, which accelerated the progression of HCC and resulted in increased patient mortality, whereas cell invasion, colony formation, proliferation, and soft agar growth were inhibited by the downregulation of RPA3 in HepG2 cells (29). It has previously been demonstrated that radiotherapy, which is an important therapeutic pathway for HCC, was able to achieve a local control rate of >90% (6). By means of irradiation, the apoptosis of SK-MES-1 cells was improved, and its growth was restrained by overexpressed miRNA-126 (27). A study has also demonstrated that migration and invasion capacity in pancreatic cancer and breast cancer was restrained by miR-146a levels, which have the potential to be applied in therapeutic treatment, and upregulation of miR-146a has been found in multiple types of cancer, such as thyroid cancer and cervical cancer (33).

Another important finding from our study was that miR-146a-5p promotes an increase in the expression of ATM, pCHK2, and Rad51 associated with the DNA repair pathway by downregulating RPA3. The previous study has found that the DNA repair pathway fell into the category of DNA damage response mechanisms, and various DNA repair pathways were activated by subnuclear DNA damage induction strategies (7). The DNA repair pathway exerted great influence on cell survival and played a role in current cancer therapeutic strategies, such as radiotherapy and cytotoxic chemotherapy (20). It has been demonstrated that the ATM kinase was activated by DNA damage, and then cellular signaling pathways of great importance were initiated (17). In addition, the previous study has shown that the Wnt/β-catenin pathway was under the regulation of miR-146a-5p through binding to the tumor suppressor Numb (13). At the same time, lines of evidence have suggested that hepatocarcinogenesis was triggered by accumulated lesions such as chromosomal aberrations and DNA damages through the DNA damage response, dysregulated DNA damage repair, and signaling-to-cell cycle checkpoints (31).

Another finding from our study revealed that the overexpression of miR-146a-5p improves radiosensitivity in HCC cells and inhibits cell proliferation. There has also been a recent report suggesting that the overexpression of miR-146a-5p resulted in the enhancement of radiosensitivity and cell apoptosis, differentiation of glioma stem cells, and reduction in the expression of stem cell marker, neurosphere formation capacity, and cell viability (32). In addition, another study revealed that the overexpression of miR-33a-5p could lead to decreased cell proliferation in WM451 cells, whereas it promoted radiosensitivity in melanoma as a result of glycolysis inhibition (4). Proliferation and activation of the hepatic stellate cell were also suppressed by overexpressed miR-146a-5p (8). Cell apoptosis was enhanced, whereas cell migration, invasion, and proliferation were restrained and cell viability reduced in HCC through miR-490-5p regulation (30). In addition, the overexpression of miR-142-5p in both SMMC-7721 and HepG2 cells resulted in the significant enhancement of cell apoptosis, whereas cell cycle arrest at the G0/G1 phase and the relative cell viability were remarkably decreased (19).

In conclusion, the findings from our study demonstrated that the overexpression of miR-146a-5p contributes to the inhibition of cell proliferation and enhancement of radiosensitivity and apoptosis in HCC by activating the DNA repair pathway and downregulation of RPA3 gene expression (Fig. 14). Further large-scale studies on miR-146a-5p and its effects on radiosensitivity in HCC would be beneficial in finding new and effective therapeutic pathways for HCC.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.L., Z.-Z.S., and T.L. conceived and designed research; T.L. and H.-L.Y. performed experiments; J.L., Z.-Z.S., T.L., and Z.-Q.Z. analyzed data; J.L., T.L., Z.-Z.S., G.-S.C., H.-Z.Q., and H.-L.Y. interpreted results of experiments; J.-Q.L. and H.-Z.Q. prepared figures; J.L., H.-Z.Q., and H.-L.Y. drafted manuscript; Z.-Z.S. and J.-Q.L. edited and revised manuscript; J.L., Z.-Z.S., T.L., J.-Q.L., Z.-Q.Z., G.-S.C., H.-Z.Q., and H.-L.Y. approved final version of manuscript.

REFERENCES

1. Bi T, Jin F, Wu W, Long J, Li Y, Gong X, Luo X, Li Z, He Q, Qu B. [Phase II clinical trial of two different modes of administration of the induction chemotherapy for locally advanced nasopharyngeal carcinoma]. Zhonghua Zhong Liu Za Zhi 37: 676–681, 2015.
2. Bottai G, Pasculli B, Calvin GA, Santarpia L. Targeting the microRNA-regulating DNA damage repair pathways in cancer. Expert Opin Biol Ther 14: 1667–1683, 2014. doi: 10.1517/14712598.2014.950650.
3. Bruix J, Reig M, Sherman M. Evidence-based diagnosis, staging, and treatment of patients with hepatocellular carcinoma. Gastroenterology 150: 835–853, 2016. doi:10.1053/j.gastro.2015.12.041.
4. Cao K, Li J, Chen J, Qian I, Wang A, Chen X, Xiong W, Tang J, Tang S, Chen Y, Chen Y, Cheng Y, Zhou J. miRNA-33a-5p increases radiosensitivity by inhibiting glycolysis in melanoma. Oncotarget 8: 83660–83672, 2017. doi:10.18632/oncotarget.19014.
5. Cun Y, Dai N, Xiong C, Li M, Sai J, Qian C, Li Z, Wang D. Silencing of APE1 enhances sensitivity of human hepatocellular carcinoma cells to radiotherapy in vitro and in a xenograft model. PLoS One 8: e55513, 2013. doi:10.1371/journal.pone.0055513.
6. Dai XF, Ding J, Zhang RG, Ren JH, Ma CM, Wu G. Radiosensitivity enhancement of human hepatocellular carcinoma cell line SMMC-7721 by sorafenib through the MEK/ERK signal pathway. Int J Radiat Biol 89: 724–731, 2013. doi:10.1080/09553002.2013.791405.
7. Dinant C, de Jager M, Essers J, van Cappellen WA, Kanaar R, Houtsmuller AB, Vermeulen W. Activation of multiple DNA repair pathways by sub-nuclear damage induction methods. J Cell Sci 120: 2731–2740, 2007. doi:10.1242/jcs.004523.
8. Du J, Niu X, Wang Y, Kong L, Wang R, Zhang Y, Zhao S, Nan Y. MiR-146a-5p suppresses activation and proliferation of hepatic stellate cells in nonalcoholic fibrosing steatohepatitis through directly targeting Wnt1 and Wnt5a. Sci Rep 5: 16163, 2015. doi:10.1038/srep16163.
9. Du L, Yu W, Dai X, Zhao N, Huang X, Tong F, Liu F, Huang Y, Ju Z, Yang W, Cong X, Xie C, Liu X, Liang L, Han Y, Qu B. Association of DNA repair gene polymorphisms with the risk of radiation pneumonitis in lung cancer patients. Oncotarget 9: 958–968, 2017. doi:10.18632/oncotarget.22982.
10. Fitzmorris P, Shoreibah M, Anand BS, Singal AK. Management of hepatocellular carcinoma. J Cancer Res Clin Oncol 141: 861–876, 2015. doi:10.1007/s00432-014-1806-0.
11. He X, Jing Y, Wang J, Li K, Yang Q, Zhao Y, Li R, Ge J, Qiu X, Li G. Significant accumulation of persistent organic pollutants and dysregulation in multiple DNA damage repair pathways in the electronic-waste-exposed populations. Environ Res 137: 458–466, 2015. doi:10.1016/j.envres.2014.11.018.
12. Hisieh JY, Huang TS, Cheng SM, Lin WS, Tsai TN, Lee OK, Wang HW. miR-146a-5p circuitry uncouples cell proliferation and migration, but not differentiation, in human mesenchymal stem cells. Nucleic Acids Res 41: 9753–9763, 2013. doi:10.1093/nar/gkt666.
13. Hwang WL, Yang MH. Numb is involved in the non-random segregation of subcellular vesicles in colorectal cancer stem cells. Cell Cycle 15: 2697–2703, 2016. doi:10.1080/15384101.2016.1218101.
14. Jin Q, Li XJ, Cao PG. MicroRNA-26b enhances the radiosensitivity of hepatocellular carcinoma cells by targeting EphA2. Tohoku J Exp Med 238: 143–151, 2016. doi:10.1620/tjem.238.143.
miR-146a-5p TARGETING RPA3 IN HCC

C311

15. Kalogeridi MA, Zygogianni A, Kyrgias G, Kouvaris J, Chatziioannou S, Kefekis N, Koudoulias V. Role of radiotherapy in the management of hepatocellular carcinoma: A systematic review. World J Hepatol 7: 101–112, 2015. doi:10.4048/wjh.v7.i1.101.

16. Kanto JH. Current status of labetalol, the first alpha- and beta-blocking agent. Int J Clin Pharmacol Ther Toxicol 23: 617–628, 1985.

17. Kitagawa R, Kastan MB. The ATM-dependent DNA damage signaling pathway. Cold Spring Harb Symp Quant Biol 70: 99–109, 2005. doi:10.1101/sq.2005.70.002.

18. Lorenz RG, Allen PM. Direct evidence for functional self-protein/Ia-molecule complexes in vivo. Proc Natl Acad Sci USA 85: 5220–5223, 1988. doi:10.1073/pnas.85.14.5220.

19. Lou K, Chen N, Li Z, Zhang B, Wang X, Chen Y, Xu H, Wang D, Wang H. MicroRNA-142-5p overexpression inhibits cell growth and induces apoptosis by regulating FOXO in hepatocellular carcinoma cells. Oncol Res 25: 65–73, 2017. doi:10.3727/096504016X14719078133366.

20. Martinek I, Haldar K, Gaitskell K, Bryant A, Nicum S, Kehoe S, Morrison J. DNA-repair pathway inhibitors for the treatment of ovarian cancer. Cochrane Database Syst Rev (6): CD007929, 2010. doi:10.1002/14651858.CD007929.pub2.

21. Mauger-Sacca’ M, Bartucci M, De Maria R. DNA damage repair pathways in cancer stem cells. Mol Cancer Ther 11: 1627–1636, 2012. doi:10.1158/1535-7163.MCT-11-1040.

22. O’Sullivan B, Shah J. New TNM staging criteria for head and neck tumors. Semin Surg Oncol 21: 30–42, 2003. doi:10.1002/sem.10019.

23. Park JH, Jung KH, Kim SJ, Fang Z, Yan HH, Son MK, Kim J, Kang JH, Lee JE, Han B, Lim JH, Hong SS. Inhibitor HS-173 through reduction of DNA damage repair in pancreatic cancer. J Cell Mol Med 21: 2872–2883, 2017. doi:10.1111/jcmm.13200.

24. Qu C, Zhao Y, Feng G, Chen C, Tao Y, Zhou S, Liu S, Chang H, Zeng M, Xia Y. RPA3 is a potential marker of prognosis and radioresistance for nasopharyngeal carcinoma. J Cell Mol Med 21: 2872–2883, 2017. doi:10.1111/jcmm.13200.

25. Schulze K, Imbeaud S, Letouze E, Alexandrov LB, Calderaro J, Rebouissou S, Couzy G, Meiller C, Shinde J, Soysouvanh F, Calatayud AL, Pinyol R, Pelletier L, Balabaud C, Laurent A, Blanc JF, Mazzaferrro V, Calvo F, Villanueva A, Nault JC, Bioulac-Sage P, Stratton MR, Llovet JM, Zucman-Rossi J. Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. Nat Genet 47: 505–511, 2015. doi:10.1038/ng.3252.

26. Wang J, Yang T, Chen H, Li H, Zheng S. Oncogene RPA1 promotes proliferation of hepatocellular carcinoma via CDK4/Cyclin-D pathway. Biochem Biophys Res Commun 498: 424–430, 2018. doi:10.1016/j.bbrc.2018.02.167.

27. Wang XC, Du LQ, Tian LL, Wu HL, Jiang XY, Zhang H, Li DG, Wang YY, Wu HY, She Y, Liu QF, Fan FY, Meng AM. Expression and function of miRNA in postoperative radiotherapy sensitive and resistant patients of non-small cell lung cancer. Lung Cancer 72: 92–99, 2011. doi:10.1016/j.lungcan.2010.07.014.

28. Watanabe H, Okada M, Kaji Y, Satouchi M, Sato Y, Yamabe Y, Onaya H, Endo M, Sone M, Arai Y. [New response evaluation criteria in solid tumours-revised RECIST guideline (version 1.1)]. Gan To Kagaku Ryoho 36: 2405–2501, 2009.

29. Xiao W, Zheng J, Zhou B, Pan L. Replication protein A 3 is associated with hepatocellular carcinoma tumorigenesis and poor patient survival. Dig Dis 36: 26–32, 2018. doi:10.1159/000478977.

30. Xu B, Xu T, Liu H, Min Q, Wang S, Song Q. MiR-490-5p suppresses cell proliferation and invasion by targeting BUB1 in hepatocellular carcinoma cells. Pharmacology 100: 269–282, 2017. doi:10.1159/000477667.

31. Yang SF, Chang CW, Wei RJ, Shius YE, Wang SN, Yeh YT. Involvement of DNA damage response pathways in hepatocellular carcinoma. Biomed Res Int 2014: 1–18, 2014. doi:10.1155/2014/153867.

32. Yang W, Yu H, Shen Y, Liu Y, Yang Z, Sun T. MiR-146b-5p overexpression attenuates stemness and radioresistance of glioma stem cells targets by downregulating HIF1α. Oncotarget 7: 41505–41526, 2016. doi:10.18632/oncotarget.9214.

33. Yuwen DL, Sheng BB, Liu J, Wenyu W, Shu YQ. MiR-146a-5p level in serum exosomes predicts therapeutic effect of cisplatin in non-small cell lung cancer. Eur Rev Med Pharmacol Sci 21: 2650–2658, 2017.

34. Zhang LH, Zhang CY, Dai XZ, Zhang J, Zhang F. Association between miR-146a single nucleotide polymorphism and genetic susceptibility to hepatocellular carcinoma: a meta-analysis. Zhonghua Gan Bing Za Zhi 25: 749–754, 2017. doi:10.3760/cma.j.issn.1007-3418.2017.10.006.

35. Zhang X, Ye ZH, Liang HW, Ren FH, Li P, Dang YW, Chen G. Down-regulation of miR-146a-5p and its potential targets in hepatocellular carcinoma validated by a TCGA- and GEO-based study. FEBS Open Bio 7: 504–521, 2017. doi:10.1002/2211-5463.12198.

36. Zhao L, Bode AM, Cao Y, Dong Z. Regulatory mechanisms and clinical perspectives of miRNA in tumor radiosensitivity. Carcinogenesis 33: 2220–2227, 2012. doi:10.1093/carcin/bgs235.

37. Zu Y, Yang Y, Zhu J, Bo X, Hou S, Zhang B, Qiu J, Zheng J. MiR-146a suppresses hepatocellular carcinoma by downregulating TRAF6. Am J Cancer Res 6: 2502–2513, 2016.