Research Article

Impact of Amarogentin on Gastric Carcinoma Cell Multiplication, Apoptosis and Migration via circKIF4A/miR-152-3p

Zhi Tan,1 Weining Wang,1 Jin Peng,1 Zhen Zhou,1 Jia Pan,1 Aiming Peng,1 Hui Cao2,2 and Wenling Fan2

1Department of Gastroenterology, First Hospital of Changsha, Changsha, 410005 Hunan, China
2Department of Health Care, First Hospital of Changsha, Changsha, 410005 Hunan, China

Correspondence should be addressed to Hui Cao; caohui61177@163.com and Wenling Fan; fwl3188@163.com

Received 15 April 2022; Revised 25 May 2022; Accepted 28 May 2022; Published 14 June 2022

Academic Editor: Fu Wang

Objective. The active ingredients extracted from natural plants have anti-GC actions and can slow down gastric carcinoma (GC) progression. To investigate the impact of Amarogentin (AG) on GC cell multiplication, apoptosis and migration and the possible mechanisms.

Methods. qRT-PCR quantification of circKIF4A and miR-152-3p in GC tissues and normal counterparts as well as HGC-27 (human GC cell strain) and GES-1 (human gastric mucosal epithelial cell strain) was performed. HGC-27 cells were intervened by AG of various concentrations. si-NC, si-circKIF4A were further transfected into HGC-27 cells. Besides, pcDNA and pcDNA-circKIF4A were transfected into HGC-27 cells, after which 60 mmol/L AG was added for intervention. Cell multiplication, clone formation, as well as apoptosis and migration measurements were made by MTT, plate clone formation, flow cytometry and Transwell assays, respectively; Double luciferase reporter assay was performed for targeting relationship identification between circKIF4A and miR-152-3p; Western blots were carried out to measure Bax and Bcl-2 protein levels.

Results. circKIF4A increased (P < 0.05) and miR-152-3p decreased (P < 0.05) in GC tissues and cell strains. Concentration-dependently, AG intervention contributed to enhanced cell multiplication inhibitory rate, apoptosis rate, miR-152-3p expression and Bax protein level (P < 0.05), together with declined number of cell clones formed, migrating cells, circKIF4A expression and Bcl-2 protein level (P < 0.05). After transfection of si-circKIF4A, cell multiplication inhibition rate, apoptosis rate and Bax protein level enhanced (P < 0.05), while cell clones formed and migrating cells as well as Bcl-2 protein level reduced (P < 0.05). miR-152-3p can be controlled by circKIF4A; pcDNA-circKIF4A transfection antagonized AG’s effects on HGC-27 cell multiplication, clone formation, apoptosis and migration. Conclusion. AG can decrease GC multiplication, clone formation and migration and induce apoptosis via modulating circKIF4A/miR-152-3p expression.

1. Introduction

In China, gastric carcinoma (GC) is a malignancy with high prevalence and death toll [1]. In the past few decades, there has been a worldwide trend of rapid urbanization and the adoption of westernized diet [2]. The previous global analysis of colorectal cancer incidence rate and mortality in 39 countries found that the incidence rate of young people (<50 years old) is significantly higher than that of people aged over 50 [3]. Advanced GC was found in most patients once diagnosed, attributing to the relatively hidden early clinical symptoms of the disease. Surgery and chemoradiotherapy are the mainstays to treat GC, but with unsatisfactory treatment outcomes and poor patient prognosis [4, 5].

Previous studies have demonstrated the long-term use of Chinese traditional herbal medicines in treating malignant diseases [6, 7]. The active ingredients extracted from natural plants have anti-GC actions and can slow down GC progression [8, 9]. Amarogentin (AG), first reported to exert anti-carcinogenic action against cutaneous carcinoma via inducing cancer cell apoptosis, is the main active component isolated from Swertiamarin, which has been shown to promote liver cancer cell apoptosis and play an anti-liver cancer part [10–12]. However, the connection between AG and GC has been rarely reported. Except from this, circular RNAs
circRNAs are ncRNA molecules with no 5' end cap structure and 3' end polyadenylate tail structure, which show aberrant expression in tumors and serve as potential targets for tumor targeted therapies. Reportedly, circRNAs interfere with cancer progression [13] and can be biomarkers to assist in cancer diagnosis and prognosis prediction [14]. Of them, circKIF4A is indicated to be up-regulated in triple negative breast cancer and can promote cancer development [15]. Moreover, following the binding to miRNA by MREs, circRNAs can be served as the regulatory gene for miRNAs [16, 17]. Starbase prediction showed that circKIF4A had binding sites with miR-152-3p, a gene that is downregulated in GC cells and could inhibit GC cell multiplication and metastasis via up-regulating its expression [18]. However, the association between circRNAs and AG has not been reported in any kinds of cancer. Meanwhile, it remains unknown regarding the mechanism of circKIF4A/miR-152-3p axis in GC pathogenesis and development.

Accordingly, the motivation and novelty of this research project is to investigate whether AG could affect GC multiplication, apoptosis and migration via modulating circKIF4A/miR-152-3p expression.

### 2. Data and Methods

#### 2.1. Materials and Reagents

The specimens of cancer tissues and paracancerous counterparts of 57 GC cases visited and treated during the period of January 2020 and April 2020 were collected and stored in a cryogenic refrigerator at -80°C. Composed of 37 males and 20 females, all patients were pathologically diagnosed as GC, with an age ranging from 50 to 66 years (mean: 58.32 ± 4.16). Informed consent of patients or their close relatives was obtained, and this study met the relevant requirements of World Medical Association Declaration of Helsinki.

AG (Chengdu Alfa Biotech); Human GC cells HGC-27 and human gastric mucosal epithelial cellsGES-1 (ATCC); DMEM, trypsin, and fetal bovine serum (FBS) (Shanghai Beyotime Biotech); Trizol reagent and Lipofectamine™ 3000 Transfection Reagent (Invitrogen, USA); Reverse Transcription and Fluorescence Quantitative PCR (Thermo Fisher, USA); si-NC, si-circKIF4A, miR-NC, and miR-152-3p mimics (Guangzhou Ruibo Bio); pcDNA, pcDNA-circKIF4A (Shanghai Genomeditech); MTT reagent, apoptosis detection kit and Transwell chamber (Beijing Solarbio); Double luciferase reporter gene vector and its activity detection kit (Promega, USA); Rabbit anti-human Bax, Bcl-2 antibodies and HRP labeled goat anti-rabbit IgG secondary antibody (CST, USA).

#### 2.2. Experimental Grouping

After seeding HGC-27 cells onto the wells of 6-well plates (1 × 10⁵ cells/well), they were cultivated in DMEM comprising AG of various concentrations (15, 30, and 60 mmol/L) for 24 h [19] and grouped as AG-L, -M and -H groups, respectively. Normal cultured HGC-27 cells were recorded as control group. Lipofectamine™

### Table 1: Sequences of primers.

| Objects        | Primer sequences                                      |
|----------------|-------------------------------------------------------|
| circKIF4A      | Forward: 5'-GAGGTACCCTGCCTGGATCT-3'                  |
|                | Reverse: 5'-TGGAATCTCCTGTAGGGCACA-3'                 |
| miR-152-3p     | Forward: 5'-GGCTTCGGCAGCACATATATAAAT-3'              |
|                | Reverse: 5'-GCCTTCAGAATTTGCGTGTCAT-3'                |
| U6             | Forward: 5'-GCTTCGGCAGCACATATATAAAT-3'               |
|                | Reverse: 5'-GCCTTCAGAATTTGCGTGTCAT-3'                |
| GAPDH          | Forward: 5'-GGAGCGAGATCCCTCCAAAAT-3'                 |
|                | Reverse: 5'-GGCTGTTGTCATACTTCTCATGG-3'               |

Figure 1: circKIF4A and miR-152-3p levels. a: circKIF4A and miR-152-3p expression in GC. b: circKIF4A and miR-152-3p expression in GC cells. *** P < 0.001.
3000 was used for the transfection. si-NC and si-circKIF4A groups were established by transfecting si-NC and si-circKIF4A into HGC-27 cells, respectively. Also, pcDNA and pcDNA-circKIF4A were transfected into HGC-27 cells. Following successful operation, they were cultured in DMEM with 60 mmol/L AG for 24 h and set as AG-H + pcDNA and AG-H + pcDNA-circKIF4A groups, respectively.

2.3. circKIF4A And miR-152-3p Detection by qRT-PCR. GC tissues, adjacent counterparts, as well as GES-1 and HGC-27 cells were added with 1 ml Trizol reagent to extract total RNA, after which cDNA synthesis was conducted by reverse transcription. The volume of qRT-PCR amplification reaction system was 20 µL, comprising 10 µL SYBR Green Master Mix, 0.8 µL forward primers, 2 µL cDNA, 0.8 µL reverse primers, and ddH2O. The mixture was pre-denatured (95°C/2 min), denatured (95°C/15 s), annealed (60°C/30 s) and extended (72°C/30 s) for 40 cycles. Levels of circKIF4A relative to GAPDH and miR-152-3p to U6 were obtained via the 2⁻ΔΔCt method. See Table 1 for primers used.

2.4. MTT Detection of Cell Multiplication. After planting HGC-27 cells into the wells of 96-well plates (3 × 10³ cells/well) and adding 20 µL MTT solution to each well, cell
culture was carried out in a 5% CO₂ and 37°C incubator for 4 h to discard the supernatants. Following the addition of 150 μL DMSO into each well, cells were cultivated (5 min) at indoor temperature without light. The came the detection of each well’s absorbance (A)₄₉₀ nm with a microplate reader (Bio-Tek, Vermont, USA) and cell multiplication inhibition rate calculation: [(A of control group - A of experimental group)/(A of control group - A of blank group)]×100%.

2.5. Plate Clone Formation Experiment. In each group, HGC-27 cells were cultured in the incubator for 14 days after inoculating them into six-well plates (1 × 10⁵/well), with the culture medium changed every other day. After discarding the culture medium and rinsing with precooled PBS, the cells were processed for 500 μL 4% paraformaldehyde immobilization (20 min), 400 μL 1% crystal violet staining (15 min), distilled water rinsing and drying, for the final microscopic (Olympus, Tokyo, Japan) counting of the number of cell clones. Five randomly chosen fields were taken by microscope to count the number of colonies with more than 10 cells, and the mean was taken.

2.6. Flow Cytometry Detection of Apoptosis Rate. The HGC-27 cells collected from each group were subjected to 0.25% trypsin digestion and centrifugation (3000 r/min, 6 min) to collect cell precipitate for subsequent rising with precooled PBS, re-suspension with 500 μL binding buffer, and incubation with AnnexinV-FITC and PI (5 μL each, 10 min). Apoptosis rate determination was made by FACS Calibur flow cytometry (FACSCalibur, BD Biosciences).

2.7. Transwell Assay for Cell Migration. HGC-27 cells were gathered for inoculation in the apical chamber (1 × 10⁵ cells/well), and 600 μL culture solution +10% FBS was placed into the basolateral one. After 24 h of cultivation, the cells were treated with PBS washing, paraformaldehyde fixation (20 min), and 0.1% crystal violet dyeing (10 min), for microscopical observation of migrating cells.

2.8. Detection of the Targeting Relationship between circKIF4Aand miR-152-3p via Double Luciferase Reporter Assay. The molecular cloning method was utilized to clone the binding loci of circKIF4A and miR-152-3p into pGL3 plasmid to build a wild-type vector WT-circKIF4A. Besides, the binding loci were mutated by gene mutation technology to construct a mutant vector MUT-circKIF4A containing the mutation site. Using lipofection, WT-circKIF4A and MUT-circKIF4A were simultaneously transfected into HGC-27 with miR-NC or miR-152-3p mimics, respectively, and then placed in an incubator for 48 h of further culture. Cells’ luciferase activity was measured after cell collection.

2.9. Western Blotting of Bax and Bcl-2 Protein Expression. HGC-27 from each group were gathered and immersed in 500 μL RIPA lysate for total protein isolation, after which the BCA method was utilized to quantify its concentration. 40 μg protein specimens were subjected to SDS-PAGE, membrane transfer, and 2 h of sealing with 5% defatted milk. This was followed by culture (4°C, 24 h) with Bax (1:1000), Bcl-2 (1:1000) primary antibodies and internal reference GAPDH antibody (1:30) diluent, as well as the subsequent incubation (37°C, 2 h) with secondary antibody diluent (1:5000). Protein bands were quantified using Quantity One software.

2.10. Statistical Processing. Data analysis adopted SPSS21.0 (IBM, Chicago, IL) and P < 0.05 was the threshold of significance. Quantitative data were expressed by (x̄ ± s), and the differences between groups and among multiple groups used independent samples t test and one-way ANOVA plus Bonferroni post hoc test, respectively.

Table 2: Impacts of Amarogentin (AG) on HGC-27 multiplication, migration and apoptosis (x ± s, n = 9).

| Group     | Control | AG-L   | AG-M   | AG-H   | F         | P         |
|-----------|---------|--------|--------|--------|-----------|-----------|
| Inhibition rate/% | 0.00 ± 0.00 | 21.64 ± 0.90* | 41.29 ± 1.90* | 59.89 ± 2.69*±# | 2046.822  | 0.000     |

Note: * P < 0.05 vs. control group; # P < 0.05 vs. AG-L group; & P < 0.05 vs. AG-M group.

Figure 3: Impact of AG on circKIF4A and miR-152-3p expression in HGC-27: a: circKIF4A in HGC-27. b: miR-152-3p in HGC-27. *P < 0.05 vs. control group; # P < 0.05 vs. AG-L group; & P < 0.05 vs. AG-M group.
3. Results

3.1. circKIF4A And miR-152-3p Expression in GC and Cells.

Compared with adjacent counterparts, circKIF4A increased in GC tissues ($P < 0.05$) while miR-152-3p decreased ($P < 0.05$), as shown in Figure 1(a); Similarly, elevated circKIF4A and reduced miR-152-3p were found in HGC-27 cells ($P < 0.05$), versus GES-1 cells, as shown in Figure 1(b).

![Figure 4: Impact of inhibiting circKIF4A on HGC-27 apoptosis, clone formation and migration]

(a) Impact of inhibiting circKIF4A on HGC-27 apoptosis.
(b) Impact of inhibiting circKIF4A on HGC-27 clone formation number.
(c) Impact of inhibiting circKIF4A on HGC-27 migration.
(d) Impact of inhibiting circKIF4A on Bax and Bcl-2 protein in HGC-27. **P < 0.001.

Table 3: Detection of impacts of inhibiting circKIF4A on HGC-27 multiplication, migration and apoptosis ($x \pm s, n = 9$).

| Group          | circKIF4A | Inhibition rate/% |
|----------------|-----------|-------------------|
| Si-NC          | 1.00 ± 0.00 | 0.00 ± 0.00       |
| Si-circKIF4A   | 0.34 ± 0.03* | 52.89 ± 1.84*     |
| t              | 66.000     | 86.234            |
| P              | 0.000      | 0.000             |

Note: * $P < 0.05$ vs. Si-NC group.
3.2. Impact of AG on HGC-27 Multiplication, Migration and Apoptosis. Concentration-dependently, HGC-27 cells in AG-L, M, and H groups presented elevated cell multiplication inhibition rate, apoptosis and Bax protein level than control cells (P < 0.05), with reduced number of cell clones formed and migrating cells as well as Bcl-2 protein (P < 0.05), as shown in Figure 2 and Table 2.

3.3. AG’s Impact on circKIF4A and miR-152-3p Expression in HGC-27. Concentration-dependently, AG-L, M and H groups showed reduced circKIF4A (P < 0.05) and elevated miR-152-3p (P < 0.05) than the control group, as shown in Figure 3.

3.4. Impact of Inhibiting circKIF4A on HGC-27 Cell Multiplication, Migration and Apoptosis. Increases in cell multiplication inhibition rate, apoptosis rate and Bax protein level (P < 0.05), as well as reductions in cell clone formation number, migrating cell number, and Bcl-2 protein level (P <0.05) were observed in si-circKIF4A group versus si-NC group, as shown in Figure 4 and Table 3.

3.5. circKIF4A Targets miR-152-3p. circKIF4A and miR-152-3p have complementary sequences, as shown in Figure 5(a). Overexpressing miR-152-3p decreased wild-type vector WT-circKIF4A luciferase activity (P <0.05; Figure 5(b)), indicating the presence of targeted regulation between circKIF4A and miR-152-3p. miR-152-3p showed up-regulated levels in si-circKIF4A group versus si-NC group (P < 0.05), and in pcDNA group versus pcDNA-circKIF4A group (P < 0.05), as shown in Figure 5(c).

3.6. Impact of circKIF4A on AG-Intervened HGC-27 Multiplication, Migration and Apoptosis. In comparison with AG-H + pcDNA group, AG-H + pcDNA-circKIF4A group had decreased cell multiplication inhibition rate, apoptosis rate and Bax protein level (P < 0.05), but increased cell clones formed, migrating cells, and Bcl-2 protein level (P < 0.05), as shown in Figure 6 and Table 4.

4. Discussion

Some traditional Chinese medicines have anti-GC actions and can play an anti-GC role by modulating multiple genes and signal axises [20]. circRNAs are reported to be either up-regulated or down-regulated in GC, and can be miRNAs’ sponge molecules and positively modulate their target genes’ expression, thus regulating the biological behavior of GC cells. circRNAs are shown to play the role of oncogene or anti-oncogene in the genesis and development of GC, which may also be potential targets for targeted therapies of the disease [21, 22]. However, whether they can be candidate targets for traditional Chinese medicine treatment of GC has not been clarified.

AG belongs to secoiridiod glycosides with anti-tumor effects [10, 23], with the ability to inhibit angiogenesis of hepatoma cells [24]. However, its role in the biological behavior of GC cells remains unknown. This work showed increased multiplication inhibition rate of GC cells and reduced number of cell clones after AG treatment, suggesting that AG can inhibit GC cell multiplication and clone formation. Up-regulating Bcl-2 can suppress apoptosis, while increasing Bax expression promotes apoptosis [25]. This study revealed that with the increase of AG concentration, the apoptosis rate of GC cells and Bax protein level increased, while Bcl-2 protein decreased, suggesting that AG can promote GC cell apoptosis. Besides, AG reduced the ability of GC cells to
Figure 6: Impact of circKIF4A on Amarogentin (AG)-intervened HGC-27 cell apoptosis, clone formation and migration. a: Impact of circKIF4A on AG-intervened HGC-27 apoptosis. b: Impact of circKIF4A on AG-intervened HGC-27 clone formation number. c: Impact of circKIF4A on AG-intervened HGC-27 migration number. d: Impact of circKIF4A on Bax and Bcl-2 protein in AG-intervened HGC-27. *** P <0.001.
migrate, suggesting the suppression of AG on GC cell migration. Apoptosis, a primary conservative process of cell clearance, is an approach through which most anticancer drugs affect cancer cells, namely, by inducing the apoptosis network of carcinoma cells [26, 27]. Similar reports showed that in the mouse liver cancer model, AG induced liver cancer cell apoptosis via up-regulating the ratio of Bax/Bcl-2, triggering the cleavage of Caspase-3 and poly adenosine diphosphate ribose polymerase [28].

circKIF4A is highly expressed in ovarian carcinoma and can promote cancer cell multiplication and metastasis [29]. It is also up-regulated in bladder cancer and can promote carcinoma cell metastasis [30]. Herein, circKIF4A in GC tissues and cells increased, and AG could concentration-dependently decrease circKIF4A levels, suggesting that AG might play an anti-GC role by inhibiting circKIF4A. Besides, we preliminarily confirmed that circKIF4A could target miR-152-3p. circRNAs have been reported to be miRNA sponges [31, 32]. In hepatocellular carcinoma, for example, circmt63 serves as miR-9 sponges [33]. And circp63 up-regulates FoxM1 expression as miR-152-3p. circRNAs have been reported to be miRNA sponges [31, 32]. In hepatocellular carcinoma, for example, circmt63 serves as miR-9 sponges to block tumor progression [33]. And circp63 up-regulates FoxM1 expression as ceRNA and promotes tumor progression in lung cancer [34]. This research demonstrates the ability of circKIF4A to interact directly with miR-152-3p. In GC cells, miR-152-3p was reportedly to be down-regulated, and up-regulating its level can inhibit cancer growth [35]. miR-152-3p overexpression can also suppress colorectal cancer cell migration and invasiveness [36]. This study identified decreased miR-152-3p in GC tissues and cells, which can be promoted by AG. Reductions in GC multiplication, clone formation, apoptosis and migration were observed after circKIF4A inhibition, while the overexpression of circKIF4A decreased AG’s impacts on GC cell multiplication, clone formation, apoptosis and migration. All these suggest that AG can promote miR-152-3p expression via inhibiting circKIF4A, thus playing an anti-gastric cancer role.

This work still has limitations. The clinical implications of AG and circKIF4A in GC need further elucidation. Besides, it is necessary to find ideal tumor markers that can be applied to diagnose GC in the early stage. Moreover, the specific pathway involved in the primary mechanism by which AG suppresses GC cell multiplication and induces apoptosis deserves investigation. Thus, more experiments need to be conducted in the future, as well as more clinical research to find out the crucial role of circKIF4A in GC, and investigate the treatment value of AG.

### Table 4: Detection of impacts of circKIF4A on Amarogentin (AG)-intervened HGC-27 multiplication, migration and apoptosis (X ± s, n = 9).

| Group                      | circKIF4A | Inhibition rate/% |
|----------------------------|-----------|-------------------|
| AG-H + pcDNA               | 1.00 ± 0.00 | 59.43 ± 4.15    |
| AG-H + pcDNA-circKIF4A     | 4.15 ± 0.13∗ | 15.15 ± 0.85∗   |
| t                          | 72.692    | 31.359            |
| P                          | 0.000     | 0.000             |

Note: ∗P < 0.05 vs. AG-H + pcDNA group.

### 5. Conclusion

To sum up, AG can inhibit GC cell multiplication, clone formation and migration, and promote apoptosis. circKIF4A is overexpressed while miR-152-3p is under-expressed in GC tissues and cells. AG can play an anti-GC role by suppressing circKIF4A to up-regulate miR-152-3p expression. Therefore, circKIF4A/miR-152-3p may serve as a candidate target of AG in treating GC. However, the specific mechanism of its action needs further exploration.

### Data Availability

The labeled dataset used to support the findings of this study are available from the corresponding author upon request.

### Conflicts of Interest

The author declare no competing interests.

### References

[1] X. Fan, X. Qin, Y. Zhang et al., “Screening for gastric cancer in China: advances, challenges and visions,” Chinese Journal of Cancer Research, vol. 33, no. 2, pp. 168–180, 2021.

[2] M. C. Wong, J. Huang, V. Lok et al., “Differences in incidence and mortality trends of colorectal cancer worldwide based on sex, age, and anatomic location,” Clinical Gastroenterology and Hepatology, vol. 19, no. 5, pp. 955–966.e61, 2021.

[3] M. C. Wong, J. Huang, P. S. Chan et al., “Global incidence and mortality of gastric cancer, 1980–2018,” JAMA Network Open, vol. 4, no. 7, article e2118457, 2021.

[4] Q. Zhang, X. Wang, S. Cao et al., “Berberine represses human gastric cancer cell growth in vitro and in vivo by inducing cyto-static autophagy via inhibition of MAPK/mTOR/p70S6K and Akt signaling pathways,” Biomedicine & Pharmacotherapy, vol. 128, article 110245, 2020.

[5] R. Xu, J. Wu, X. Zhang et al., “Modified Bu-zhong-yi-qi decoction synergises with 5 fluorouracile to inhibits gastric cancer progress via PD-1/PD-L1-dependent T cell immunization,” Pharmacological Research, vol. 152, article 104623, 2020.

[6] J. Yang, L. Zhu, Z. Wu, Y. Wang, and Cochrane Upper GI and Pancreatic Diseases Group, “Chinese herbal medicines for induction of remission in advanced or late gastric cancer,” Cochrane Database of Systematic Reviews, vol. 4, 2013.

[7] Y. K. Lee, K. Bae, H.-S. Yoo, and S.-H. Cho, “Benefit of adjuvant traditional herbal medicine with chemotherapy for resectable gastric cancer,” Integrative Cancer Therapies, vol. 17, no. 3, pp. 619–627, 2018.

[8] M. Song, X. Wang, Y. Luo et al., “Cantharidin suppresses gastric cancer cell migration/invasion by inhibiting the pi3k/akt signaling pathway via ccat1,” Chemico-Biological Interactions, vol. 317, article 108939, 2020.

[9] X. Huang, J. Qian, L. Li et al., “Curcumol improves cisplatin sensitivity of human gastric cancer cells through inhibiting pi3k/akt pathway,” Drug Development Research, vol. 81, no. 8, pp. 1019–1025, 2020.

[10] C. Huang, R. Li, Y. Zhang, and J. Gong, “Amarogentin induces apoptosis of liver cancer cells via upregulation of p53 and downregulation of human telomerase reverse transcriptase in
mice,” Technology in Cancer Research & Treatment, vol. 16, no. 5, pp. 546–558, 2017.

[11] P. Saha, S. Mandal, A. Das, P. C. Das, and S. Das, ”Evaluation of the anticarcinogenic activity of Swertia chirata Buch.Ham, an Indian medicinal plant, on DMBA-induced mouse skin carcinogenesis model,” Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, vol. 18, no. 5, pp. 373–378, 2004.

[12] P. Saha, S. Mandal, A. Das, and S. Das, ”Amarogentin can reduce hyperproliferation by downregulation of cox-ii and upregulation of apoptosis in mouse skin carcinogenesis model,” Cancer Letters, vol. 244, no. 2, pp. 252–259, 2006.

[13] J. Beermann, M.-T. Piccoli, J. Viereck, and T. Thum, ”Non-coding rnas in development and disease: background, mechanisms, and therapeutic approaches,” Physiological Reviews, vol. 96, no. 4, pp. 1297–1325, 2016.

[14] E. Arnaiz, C. Sole, L. Manterola, L. Iparraguirre, D. Otaegui, and C. H. Lawrie, ”Circrnas and cancer: Biomarkers and master regulators,” Seminars in Cancer Biology, vol. 58, pp. 90–99, 2019.

[15] H. Tang, X. Huang, J. Wang et al., ”CircRfe4a acts as a prognostic factor and mediator to regulate the progression of triple-negative breast cancer,” Molecular Cancer, vol. 18, no. 1, pp. 1–9, 2019.

[16] T. B. Hansen, T. I. Jensen, B. H. Clausen et al., ”Natural rna circles function as efficient microrna sponges,” Nature, vol. 495, no. 7441, pp. 384–388, 2013.

[17] X.-Y. Huang, Z.-L. Huang, Y.-H. Xu et al., ”Comprehensive circular rna profiling reveals the regulatory role of the cir-crna-100338/mir-141-3p pathway in hepatitis b-related hepatocellular carcinoma,” Scientific Reports, vol. 7, no. 1, pp. 1–12, 2017.

[18] P. Ma, L. Li, F. Liu, and Q. Zhao, ”Hnf1a-induced Incrarna hcg18 facilitates gastric cancer progression by upregulating dnajb12 via mir-152-3p,” Oncotargets and Therapy, vol. 13, pp. 7641–7652, 2020.

[19] X. Wu, S. Li, and S. An, ”Effects of gentiopicroside on oxidative stress and apoptosis in gastric cancer cells,” Journal of Biomaterials and Tissue Engineering, vol. 11, no. 3, pp. 553–559, 2021.

[20] Z. M. Liu, X. L. Yang, F. Jiang, Y. C. Pan, and L. Zhang, ”Matrine involves in the progression of gastric cancer through inhibiting mir-93-5p and upregulating the expression of target gene alnak,” Journal of Cellular Biochemistry, vol. 121, no. 3, pp. 2467–2477, 2020.

[21] X. Zhang, S. Wang, H. Wang et al., ”Circular rna circmirp1 acts as a microrna-149-5p sponge to promote gastric cancer progression via the akt1/mtor pathway,” Molecular Cancer, vol. 18, no. 1, pp. 1–24, 2019.

[22] M. Jie, Y. Wu, M. Gao et al., ”Circmirps35 suppresses gastric cancer progression via recruiting kat7 to govern histone modification,” Molecular Cancer, vol. 19, no. 1, pp. 1–16, 2020.

[23] K. Patel, V. Kumar, A. Verma, M. Rahman, and D. K. Patel, ”Amarogentin as topical anticancer and anti-infective potential: scope of lipid based vesicular in its effective delivery,” Recent Patents on Anti-Infective Drug Discovery, vol. 14, no. 1, pp. 7–15, 2019.

[24] Y. Zhang, Y. Zhang, J. Wang, and H. Gu, ”Amarogentin inhibits liver cancer cell angiogenesis after insufficient radio-frequency ablation via affecting stemness and the p53-dependent vegfa/dll4/notch1 pathway,” BioMed Research International, vol. 2020, Article ID 5391058, 9 pages, 2020.

[25] X. Zhang, Y. Qin, Z. Pan et al., ”Cannabidiol induces cell cycle arrest and cell apoptosis in human gastric cancer sgc-7901 cells,” Biomolecules, vol. 9, no. 8, p. 302, 2019.

[26] G. Pistruttio, D. Trisciuglio, C. Ceci, A. Garufi, and G. D’Orazi, ”Apoptosis as anticancer mechanism: function and dysfunction of its modulators and targeted therapeutic strategies,” Aging, vol. 8, no. 4, pp. 603–619, 2016.

[27] S. Vijayarathna, S. L. Jothy, Y. Chen, J. R. Kanwar, and S. Sasidharan, ”Anti-cancer natural products inducing cross-talk between apoptosis and autophagy mutual proteins to regulate cancer cell death: design of future green anticancer therapies,” Asian Pacific Journal of Cancer Prevention, vol. 16, no. 14, pp. 6175–6176, 2015.

[28] D. Pal, S. Sur, S. Mandal et al., ”Prevention of liver carcinogenesis by amarogentin through modulation of g 1/s cell cycle check point and induction of apoptosis,” Carcinogenesis, vol. 33, no. 12, pp. 2424–2431, 2012.

[29] S. Sheng, Y. Hu, F. Yu et al., ”CircRfe4a sponges mir-127 to promote ovarian cancer progression,” Aging, vol. 12, no. 18, pp. 17921–17929, 2020.

[30] Y. R. Shi, Z. Wu, K. Xiong et al., ”Circular rna circRfe4a sponges mir-375/1231 to promote bladder cancer progression by upregulating notch2 expression,” Frontiers in Pharmacology, vol. 11, 2020.

[31] L. S. Kristensen, M. S. Andersen, L. V. Stagsted, K. K. Ebbesen, T. B. Hansen, and J. Kjems, ”The biogenesis, biology and characterization of circular rnas,” Nature Reviews Genetics, vol. 20, no. 11, pp. 675–691, 2019.

[32] F. Ye, G. Gao, Y. Zou et al., ”Circfbxw7 inhibits malignant progression by sponging mir-197-3p and encoding a 185-aa protein in triple-negative breast cancer,” Molecular Therapy–Nucleic Acids, vol. 18, pp. 88–98, 2019.

[33] D. Han, J. Li, H. Wang et al., ”Circular rna circmto1 acts as the sponge of microrna-9 to suppress hepatocellular carcinoma progression,” Hepatology, vol. 66, no. 4, pp. 1151–1164, 2017.

[34] Z. Cheng, C. Yu, S. Cui et al., ”c _ircTP63_ functions as a ceRNA to promote lung squamous cell carcinoma progression by upregulating FOXM1,” Nature Communications, vol. 10, no. 1, pp. 1–13, 2019.

[35] X. Wang, Y. Zhang, W. Li, and X. Liu, ”Knockdown of cir_ RNA PVT1 Elevates Gastric Cancer Cisplatin Sensitivity via Sponging mir-152-3p,” Journal of Surgical Research, vol. 261, pp. 185–195, 2021.

[36] L. B. Sun, S. F. Zhao, J. J. Zhu, Y. Han, and T. D. Shan, ”Long noncoding rna ucid sponges mir-152-3p to promote colorectal cancer cell migration and invasion via the wnt/β-catenin signaling pathway,” Oncology Reports, vol. 44, no. 3, pp. 1194–1205, 2020.