Knockdown of lncRNA X inactive specific transcript (XIST) radiosensitizes non-small cell lung cancer (NSCLC) cells through regulation of miR-16-5p/WEE1 G2 checkpoint kinase (WEE1) axis

Ran Du1#, Feng Jiang2#, Yanhua Yin1, Jinfen Xu3, Xia Li3, Likuan Hu4 and Xiuyu Wang1

Abstract
Long non-coding RNA (IncRNA) X inactive specific transcript (XIST) is reported to play an oncogenic role in non-small cell lung cancer (NSCLC). However, the role of XIST in regulating the radiosensitivity of NSCLC cells remains unclear. Quantitative real-time polymerase chain reaction (qRT-PCR) was used to detect the expressions of XIST and miR-16-5p in NSCLC in tissues and cells, and Western blot was used to assess the expression of WEE1 G2 checkpoint kinase (WEE1). Cell counting kit-8 (CCK-8), colony formation and flow cytometry assays were used to determine cell viability and apoptosis after NSCLC cells were exposed to different doses of X-rays. The interaction between XIST and miR-16-5p was confirmed by StarBase database, qRT-PCR and dual-luciferase reporter gene assays. TargetScan database was used to predict WEE1 as a target of miR-16-5p, and their targeting relationship was further validated by Western blot, qRT-PCR and dual-luciferase reporter gene assays. XIST was highly expressed in both NSCLC tissue and cell lines, and knockdown of XIST repressed NSCLC cell viability and cell survival, and facilitated apoptosis under the irradiation. MiR-16-5p was a target of XIST, and rescue experiments demonstrated that miR-16-5p inhibitors could reverse the role of XIST knockdown on radiosensitivity in NSCLC cells. WEE1 was validated as a target gene of miR-16-5p, and WEE1 could be negatively regulated by XIST. XIST promotes the radioresistance of NSCLC cells by regulating the expressions of miR-16-5p and WEE1, which can be a novel target for NSCLC therapy.

Keywords
MiR-16-5p, NSCLC, radiosensitivity, WEE1, XIST

Introduction
Lung cancer is one of the leading causes of cancer-related deaths in the world, and non-small cell lung cancer (NSCLC) accounts for more than 80% of all lung cancer cases.1–3 Radiation therapy is an important treatment strategy for advanced NSCLC patients.4,5 However, radiation resistance has become a serious obstacle to the effects of radiotherapy.6–8 To improve the clinical outcomes of NSCLC therapy, it is urgent to further explore the mechanisms by which NSCLC cells develop radioresistance.

1Department of Pathology, Liaocheng People’s Hospital, Liaocheng, Shandong, China
2Department of Thoracic surgery, Liaocheng Tumor Hospital, Liaocheng, Shandong, China
3Department of Oncology, Laigang Hospital Affiliated to Taishan Medical University, Laiwu, Shandong, China
4Department of Radiation and Oncology, Qilu Hospital, Shandong University, Jinan, Shandong, China
#co-first author: Ran Du, Feng Jiang

Corresponding author:
Xiuyu Wang, Department of Pathology, Liaocheng People’s Hospital, No. 67 Dongchang West Road, Liaocheng 252000, Shandong, China. Email: wxy830205@163.com
Long non-coding RNAs (lncRNAs) are recognized as a new set of clinical biomarkers and potential tumor therapeutic targets. LncRNAs are a class of non-protein-coding transcripts with a length of longer than 200 nucleotides. They are involved in the regulation of various biological processes including cell proliferation, migration and apoptosis, as well as the progression of cancer. Abnormally expressed lncRNAs participate in the progression of NSCLC. For example, LINC01296 promotes the progression of NSCLC by sponging miR-5095. LncRNA AK027294 facilitates NSCLC progression by up-regulating the expression of STAT3. In addition, lncRNA X inactive specific transcript (XIST) is proved to promote the development and progression of NSCLC. In terms of mechanism, XIST is a molecular sponge of miR-141, miR-367 and miR-137, and up-regulated expression of XIST results in decreased expression of these microRNAs (miRNAs) and in turn promotes the proliferation and metastasis of cancer cells. It is reported that overexpression of XIST in nasopharyngeal carcinoma cells can down-regulate the expression of miR-29c and inhibit the radiosensitivity of tumor cells. However, little is known about the role of XIST in NSCLC radiosensitivity.

MiRNAs are a class of small non-coding RNAs containing 18–25 nucleotides, leading to translational inhibition or mRNA degradation by specifically binding to the 3'-untranslated region (3'-UTR) of the target mRNA. The abnormal expression of miRNAs is closely related to carcinogenesis, playing a critical role in various biological processes such as cell differentiation, stress response, proliferation, apoptosis and so on. As one of the miRNAs associated with human malignancies, miR-16-5p promotes tumor cell radiosensitivity by modulating the Cyclin D1/E1–pRb–E2F1 signaling in prostate cancer cells. However, whether miR-16-5p promotes the radiosensitivity of NSCLC cells and its underlying mechanisms remain unknown.

WEE1 G2 checkpoint kinase (WEE1) is a tyrosine kinase that exhibits the regulatory function in the G2 checkpoint in response to DNA damage. Previous studies confirm that WEE1 is a potential target for tumor therapy, such as gastric cancer, acute myelocytic leukemia, head and neck squamous cell carcinomas and melanoma. WEE1 expression is up-regulated in these malignancies and is closely associated with the adverse prognosis of the patients. In addition, inhibition of WEE1 expression is reported to enhance the radiosensitivity of osteosarcoma and pancreatic cancer. A recent study indicates that the WEE1 inhibitor, AZD1755, sensitizes KRAS mutant NSCLC cells to radiotherapy. However, the role of WEE1 in NSCLC radioresistance and its upstream regulatory mechanism have not been fully elucidated.

In this study, bioinformatics analysis indicated that XIST had a potential binding site with miR-16-5p and WEE1 was a downstream target of miR-16-5p. We investigated the role of XIST in the radioresistance of NSCLC cells in vitro and its function of regulating WEE1 by sponging miR-16-5p, providing a theoretical basis for the treatment of NSCLC.

Materials and methods

Clinical samples

All patients enrolled signed informed consent in this study, and our research was endorsed by the Ethics Committee of Qilu Hospital (Approval number: 201705006). 31 cases of NSCLC tissues (13 squamous cell carcinomas and 18 adenocarcinomas) and adjacent normal tissues were taken from the Department of Pathology, Qilu Hospital. All patients were diagnosed as NSCLC by histopathology and had never received preoperative chemotherapy or radiation therapy before this study.

Cell lines and cell culture

Human lung cancer cell lines (H157, HCC827, A549 and H838) and normal bronchial epithelial cell lines (16HBE) were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). All cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Hyclone, Logan, UT, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Invitrogen, Grand Island, NY, USA), 100U/ml penicillin and 100 μg/ml streptomycin (Gibco, Carlsbad, CA, USA) in an incubator at 37°C in 5% CO₂.

Cell transfection

Small interference RNA (siRNA) control (si-con), siRNAs against XIST (si-XIST-1 and si-XIST-2), pcDNA3.1 vector (vector), pcDNA3.1-XIST, miRNA control (miR-con), miR-16-5p mimics (miR-16-5p),
and miR-16-5p inhibitors (anti-miR-16-5p) were available from GenePharma Co., Ltd. (Shanghai, China). H838 and A549 cells were seeded in 6-well cell culture plates at a density of $1 \times 10^5$ /mL and transfected with the siRNAs (50nmol), mimics (20nmol), or inhibitors (20nmol) using Lipofectamine® 3000 (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the supplier’s instructions. Transfection efficiency was measured by quantitative real-time polymerase chain reaction (qRT-PCR).

Ionizing radiation treatment
Transfected NSCLC cells were irradiated with a linear accelerator (Varian Medical Systems, USA) at room temperature with different doses (0, 2, 4, 6, and 8 Gy, dose rate: 1 Gy/min). After 24–96 h, the cells were used for further analyses.

qRT-PCR
Total RNA from tissues and cells was extracted using TRizol reagent (Invitrogen, Shanghai, China). 1 μg of total RNA was reversely transcribed into complementary DNA (cDNA) using SuperScript First-Strand Synthesis System (Invitrogen, Shanghai, China). Then qRT-PCR was performed with SYBR Green Master Mix (Takara, Dalian, China). The relative expressions of XIST and miR-16-5p were calculated employing the $2^{-\Delta\Delta Ct}$ method. Additionally, to determine the subcellular fractionation location of lncRNA, Cytoplasmic & Nuclear RNA Purification kit (Takara, Hefei, China) was used to obtain the cytoplasmic and nuclear RNA of the cells, respectively. The primers used were as follows: XIST: 5'-GCATAACTCGGCTTAGGGCT-3' (forward) and 5'-TCCTCTGCCTGACCTGCTAT-3' (reverse); miR-16-5p, 5'-TAGCAGCACGTAAATATTGGCG-3' (forward) and 5'-TGCGTGTCGTGGAGTC-3' (reverse); WEE1: 5'-GCTGCCTCTGAAGAAGGAGA-3' (forward); 5'-ACATACCACTGTGAGGGCAA-3' (reverse); U6, 5'-CTCGCTTCGGCAGCA-3' (forward) and 5'-AACGCTTCACGAATTTGCGT-3' (reverse); β-actin, 5'-CGTGAAAAGATGACCCAGATCA-3' (forward) and 5'-CAGCCTGGAATTGGCTACGTACA-3' (reverse)

Western blot
The cells were lysed with RIPA lysis buffer (Beyotime Biotechnology, Shanghai, China). The supernatant was collected after centrifugation. After the protein was quantified by Enhanced BCA Protein Assay Kit (Beyotime, Shanghai, China), SDS-PAGE was performed, and then the protein was transferred onto the PVDF membrane (Life Technologies, Gaithersburg, MD, USA). Then WEE1 antibody (Abcam, ab137377, 1:1000) and internal reference β-actin antibody (Abcam, ab20272, 1:1000) were added, with which the membranes were incubated overnight at 4°C. After the PVDF membrane was washed with TBST solution, they were incubated with the horseradish peroxidase-labeled secondary antibody (Hubei Biossc Biotechnology Co. Ltd., 1:2000) for 1 h at room temperature. After the membranes were rinsed with TBST solution again, the hypersensitive ECL (Hubei Biossci Biotechnology Co., Ltd.) was used for chemiluminescence, and the protein bands were developed.

Cell viability assay
H838 and A549 cells in the logarithmic growth phase were selected and then trypsinized. In brief, 100 μL cell suspension containing $2 \times 10^3$ cells was added into each well of the 96-well plates. Then the 96-well plates were placed in an incubator to continue the culture. After 24 h, 10 μL of cell counting kit 8 (CCK-8) solution (Biossci, Wuhan, China) was added to each well and incubated for another hour in the incubator. After the end of the culture, the 96-well plates were placed in a microplate reader, and the absorbance (optical density, OD value) of each well at a wavelength of 450 nm was measured. Thereafter, the absorbance of the cells was measured at 48, 72, and 96 h, respectively.

Colony formation experiment
Transfected NSCLC cells were seeded into 6-well plates ($1 \times 10^3$ / well) and irradiated with the indicated single dose (0, 2, 4, 6 or 8 Gy). After 2 weeks of culture, cells were fixed with 100% methanol and stained with 0.1% crystal violet for 15 min. The number of colony in each group was then counted and recorded with naked eyes.

Flow cytometry
Cells in each group were treated with or without 4 Gy irradiation, and the percentage of the apoptotic cells was examined by a FACScalibur Flow Cytometer (BD Biosciences, San Jose, CA, USA)
using Annexin V-FITC / propidium iodide (PI) double staining kit (Invitrogen, Shanghai, China).

**Luciferase reporter assay**

The wild type (WT) 3′UTR of WEE1 (WEE1-WT-3′UTR) or the sequence of XIST containing the predicted miR-16-5p targeting site (XIST -WT) was amplified, and then inserted into pGL3 basic vector (Promega, Madison, WI, USA). Similarly, the sequence of mutant (MUT) WEE1 3′UTR or XIST was inserted into the luciferase reporter vector to obtain WEE1-MUT-3′UTR and XIST-MUT reporter plasmids. The cells were seeded in 24-well plates at 5000 cells per well, and cultured for 24 h. After that, the cells were co-transfected with the reporter plasmids with miR-16-5p mimics or miR-con. 48 h later, the relative luciferase activity of each group was determined using dual-luciferase reporter gene assay system (Promega, Madison, WI, USA).

**Statistical analysis**

All experiments were repeated at least three times independently and the results were shown as mean ± standard deviation (SD). Statistical differences between the two groups were assessed using Student’s t-test. One-way analysis of variance followed by Tukey’s post hoc test was used to compare differences among multiple groups. \( P < 0.05 \) was considered statistically significant.

**Results**

**XIST expression was up-regulated in NSCLC tissues and cell lines, and associated with the radioresistance of NSCLC cells**

First of all, qRT-PCR was used to examine the expression of XIST in 31 cases of NSCLC tissues and adjacent tissues. It was found that XIST was significantly highly expressed in NSCLC tissues compared with in adjacent normal tissues (Figure 1(a)). Subsequently, the expression of XIST in four different NSCLC cell lines was detected by qRT-PCR. Compared with in the normal bronchial epithelial cell line 16HBE, XIST was significantly highly expressed in all of the four NSCLC cell lines including H157, HCC827, H838, and A549 (Figure 1(b)). To further delve into the role of XIST in the development of radiotherapy resistance in NSCLC, we transfected siRNAs targeting XIST into H838 and A549 cells, and qRT-PCR results confirmed that we successfully constructed cell models with low expression of XIST (Figure 1(c)). Under different doses of radiation, we detected the colony formation of H838 and A549 cells employing colony formation assay. The results suggested that XIST knockdown markedly reduced the number of colonies (Figure 1(d) and (e)). CCK-8 assay confirmed knockdown XIST significantly inhibited the viability of NSCLC cell compared with the control group (Figure 1(f)). Knockdown of XIST also promoted NSCLC cell apoptosis in the absence or presence of radiation (4 Gy) (Figure 1(g) and (h)). Collectively, these data indicated that XIST knockdown enhanced the radio-sensitivity of NSCLC cells.

**XIST functioned as competing endogenous RNA and sponged miR-16-5p in NSCLC**

The online bioinformatics database StarBase was used to search for potential miRNAs capable of pairing with XIST. It was found that XIST had a putative binding site for the seed sequence of miR-16-5p (Figure 2(a)). To further validate whether XIST could sequester miR-16-5p, dual-luciferase reporter assays were performed, and the results of which revealed that miR-16-5p mimics inhibited the luciferase activity of the XIST-WT reporter, but didn’t affect that of the XIST-MUT reporter (Figure 2(b)). Additionally, XIST overexpression decreased miR-16-5p expression while XIST knockdown worked oppositely in NSCLC cells (Figure 2(c)). Besides, XIST was preferentially localized in the cytoplasm of NSCLC cells, which was determined by subcellular fractionation assay, and this suggested that XIST could probably function as a competing endogenous RNA (ceRNA) (Figure 2(d)). Moreover, Pearson’s correlation analysis unmasked a negative correlation between miR-16-5p and XIST expressions in NSCLC tissues (Figure 2(e)). Taken together, we concluded that miR-16-5p was a down-stream target of XIST and its expression level was negatively regulated by XIST.

**XIST and miR-16-5p showed opposite changes in NSCLC under irradiation**

Subsequently, our data showed that miR-16-5p expression was down-regulated in NSCLC tissues and cell lines compared to in adjacent tissues and
16HBE cell line (Figure 3(a) and (b)). To further investigate whether radiation exposure would cause changes in the expressions of XIST and miR-16-5p in H838 and A549 cells, their expressions in H838 and A549 cells were detected by qRT-PCR at the interval of 3 h after the cells were treated with 4 Gy...
radiation. It was authenticated that XIST expression in H838 and A549 cells was significantly increased compared with that of the control group (0 Gy) (Figure 3(c)); and as expected, miR-16-5p expression was significantly inhibited with the same treatment (Figure 3(d)). The above results indicated that XIST and miR-16-5p showed opposite changes under irradiation, and changes in the expressions of XIST and miR-16-5p might affect the radiosensitivity of NSCLC cells.

Inhibition of miR-16-5p reversed the effects of XIST knockdown on NSCLC cells

To further elaborate on the underlying mechanism by which XIST induced radioresistance of NSCLC cells, we transfected si-con, si-XIST or si-XIST+anti-miR-16-5p into H838 and A549 cells, respectively. qRT-PCR showed that the transfection was successful (Supplementary Figure 1). The results of CCK-8 assay suggested that the inhibitory effect on cell viability induced by XIST knockdown was attenuated by anti-miR-16-5p (Figure 4(a)). In addition, we treated these cells with different doses of X-rays, and as shown, XIST knockdown sensitized NSCLC cells to irradiation, increasing the apoptosis and reducing the colony formation, while anti-miR-16-5p attenuated these effects (Figure 4(b)–(d)).

MiR-16-5p directly targeted the 3’ UTR of WEE1

Next, we predicted the target gene of miR-16-5p with TargetScan database and found that WEE1 was one of the candidate target genes of miR-16-5p (Figure 5(a)). qRT-PCR analysis unearthed that...
miR-16-5p mimics was successfully transferred into NSCLC cells (Supplementary Figure 2). qRT-PCR and Western blot showed that WEE1 mRNA and protein were significantly decreased after the transfection of si-XIST (Figure 5(f)). Besides, we found a significant positive correlation between XIST and WEE1 mRNA expressions in NSCLC tissues (Figure 5(g)). These results indicated that WEE1 was a downstream gene of miR-16-5p, and its expression level was negatively regulated by the latter, and XIST could positively regulate the expression level of WEE1 indirectly.

**Discussion**

Previous studies indicate that XIST, miR-16-5p, WEE1 figure prominently in regulating the development and progression of NSCLC.\textsuperscript{17,18,38,39} It is widely accepted that radiotherapy is currently one of the most effective methods for the treatment of a variety of advanced malignancies, including NSCLC.\textsuperscript{2,40} However, the effect of radiotherapy is not satisfactory due to the reduced sensitivity of NSCLC cells to X-rays.\textsuperscript{41} In this study, we validate that XIST was up-regulated in NSCLC tissues and cell lines, while miR-16-5p was down-regulated. Additionally, we proved that XIST knockdown sensitized NSCLC cells to irradiation, and XIST could positively regulate the expression of WEE1, probably via repressing miR-16-5p. Our work provided a novel ceRNA network compose of XIST, miR-16-5p and WEE1, which participated in the radioresistance of NSCLC cells.

It is worth noting that in recent years, more and more studies confirm that non-coding RNA plays an important regulatory role in the occurrence and development of various malignant tumors.\textsuperscript{7,42–45} For example, XIST expression level is abnormally up-regulated in NSCLC, and associated with shorter survival time and worse prognosis.\textsuperscript{46} In terms of mechanism, XIST promotes the occurrence and development of NSCLC through inhibiting KLF2 expression by binding to EZH2.\textsuperscript{46} Moreover, XIST is confirmed to be an oncogene in nasopharyngeal carcinoma, and it promotes cancer progression through up-regulating E2F3 expression by sponging miR-34a-5p.\textsuperscript{47} It is also indicated that XIST knockdown inhibits proliferation of nasopharyngeal carcinoma cells and increases radiosensitivity by inhibiting DNA damage repair.\textsuperscript{21} In this study, we found XIST expression was markedly upregulated in NSCLC tissue and cell lines. The expression level of XIST in NSCLC cells was observably increased under the irradiation. Besides,
we successfully constructed NSCLC cell models with lowly expressed XIST. Knockdown of XIST significantly inhibited cell proliferation and survival, and promoted apoptosis, suggesting that XIST was a promising therapeutic target to sensitize NSCLC cells to radiotherapy.

MiRNAs are also important regulators of radiosensitivity in human tumor cells. For example, miR-16-5p targets Cyclin D1/E1 3'-UTR in prostate cancer and induces cell cycle arrest in the G0/G1 phase by modulating the Cyclin D1/Cyclin E1/pRb/E2F1 pathway; overexpression of miR-16-5p inhibits cell viability and promotes apoptosis to enhance the sensitivity to X-rays. In addition, miR-16-5p expression is reported to be significantly down-regulated in the serum of NSCLC patients. Therefore, we were curious about the role of miR-16-5p in the development of radiosensitivity in NSCLC cells and its regulatory mechanisms. In the present study, we found that miR-16-5p expression was observably down-regulated in NSCLC tissues compared with that in adjacent normal tissues. Subsequently, we predicted the presence of binding sites between XIST and miR-16-5p through an online bioinformatics database. Dual luciferase reporter assay verified that XIST could sponge miR-16-5p and negatively regulate the expression of the latter. Functional experiments manifested that the promoting effect of knockdown XIST on the radiosensitivity of NSCLC cells could be reversed by the miR-16-5p inhibitors. Therefore, we concluded that XIST reduced the radiosensitivity of NSCLC cells by modulating the expression of miR-16-5p.

In recent years, the significance of WEE1 in regulating the biological behaviors of NSCLC cells has become increasingly prominent, including its regulatory function on the radiosensitivity of cancer cells. It is reported that inhibition of WEE1 expression is a potential therapeutic approach to increase the radiosensitivity of NSCLC cells harboring p53 and KRAS mutations. In the present work, we explored the regulatory mechanism of WEE1 expression in NSCLC cells. We found that WEE1 was a downstream target of miR-16-5p, and its expression level was negatively regulated by the latter. Subsequently, we transfected si-XIST into NSCLC cells and found that the expression of WEE1 was significantly inhibited. Therefore, we
Du et al. concluded that WEE1 was directly and negatively regulated by miR-16-5p and could be positively and indirectly regulated by XIST.

**Conclusion**

In summary, knockdown of XIST enhances the radiosensitivity of NSCLC cells by modulating the miR-16-5p/WEE1 axis, and it is expected to expand the understanding of the molecular mechanisms of radioresistance of NSCLC. However, this study in which only *in vitro* experiments were conducted needs to be reconfirmed with animal models.

**Acknowledgements**

None.

**Authors’ Contribution**

Conceived and designed the experiments: JFX, LKH, XYW; Performed the experiments: JFX, XL, LKH, RD, FJ, YHY; Analyzed statistic: JFX; Wrote the paper: JFX, LKH; Performed the revision: RD, FJ, YHY.

All authors read and approved the final manuscript.

**Declaration of Conflicting Interests**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Funding**

The author(s) received no financial support for the research, authorship, and/or publication of this article.
Ethics approval
Ethical approval for this study was obtained from Ethnic Committee of Qilu Hospital (APPROVAL NUMBER: 201705006)*.

Informed consent
Written informed consent was obtained from all subjects before the study.

Trial registration
Not applicable.

ORCID iD
Likuan Hu https://orcid.org/0000-0002-6450-4633

Data Availability Statement
The data used to support the findings of this study are available from the corresponding author upon request.

Supplemental material
Supplemental material for this article is available online.

References
1. Jiang W, Jin G, Cai F, et al. (2019) Extracellular signal-regulated kinase 5 increases radioresistance of lung cancer cells by enhancing the DNA damage response. Experimental and Molecular Medicine 51(2): 19.
2. Wu D, Li Y, Zhang H, et al. (2017) Knockdown of Lncrna PVT1 enhances radiosensitivity in non-small cell lung cancer by sponging Mir-195. Cellular Physiology and Biochemistry 42(6): 2453–2466.
3. Sheng J, Wang L, Han Y, et al. (2018) Dual roles of protein as a template and a sulfur provider: A general approach to metalsulfides for efficient photothermal therapy of cancer. Small 14(1): 1702529.
4. Zheng L, Wang Y, Xu Z, et al. (2019) Concurrent EGFR-TKI and thoracic radiotherapy as first-line treatment for stage IV non-small cell lung cancer harboring EGFR active mutations. Oncologist 24(8): 1031–e612.
5. Voong KR, Hazell SZ, Fu W, et al. (2019) Relationship between prior radiotherapy and checkpoint-inhibitor pneumonitis in patients with advanced non-small-cell lung cancer. Clinical Lung Cancer 20(4): e470–e479.
6. Yin J, Zhao J, Hu W, et al. (2017) Disturbance of the let-7/LIN28 double-negative feedback loop is associated with radio- and chemo-resistance in non-small cell lung cancer. PLoS One 12(2): e0172787.
7. Zhou YL, Li Y, Luo DM, et al. (2015) Microstructures, mechanical and corrosion properties and biocompatibility of as extruded Mg-Mn-Zn-Nd alloys for biomedical applications. Materials Science and Engineering C: Materials for Biological Applications 49: 93–100.
8. Tang L, Wei F, Wu Y, et al. (2018) Role of metabolism in cancer cell radiosensitivity and radiosensitization methods. Journal of Experimental and Clinical Cancer Research 37(1): 87.
9. Talebi A, Akbari A, Mobini GR, et al. (2019) Biological and clinical relevance of long non-coding RNA PCAT-1 in cancer, a systematic review and meta-analysis. Asian Pacific Journal of Cancer Prevention 20(3): 667–674.
10. Wang S, Zhang S, He Y, et al. (2019) HOXA11-AS regulates JAK-STAT pathway by miR-15a-3p/STAT3 axis to promote the growth and metastasis in liver cancer. Journal of Cellular Biochemistry 120(9): 15941–15951.
11. Bhan A, Soleimani M and Mandal SS. (2017) Long noncoding RNA and cancer: A new paradigm. Cancer Research 77(15): 3965–3981.
12. Wang KC, Yang YW, Liu B, et al. (2011) A long noncoding RNA maintains active chromatin to coordinate homeotic gene expression. Nature 472(7341): 120–124.
13. Zhou J, Wang C, Gong W, et al. (2018) uc.454 Inhibited growth by targeting heat shock protein family a member 12B in non-small-cell lung cancer. Molecular Therapy: Nucleic Acids 12: 174–183.
14. Zhang L, Hu J, Li J, et al. (2019) Long noncoding RNA LINC-PINT inhibits non-small cell lung cancer progression through sponging miR-218-5p/PDCD4. Artificial Cells, Nanomedicine, and Biotechnology 47(1): 1595–1602.
15. Hu X, Duan L, Liu H, et al. (2019) Long noncoding RNA LINC01296 induces non-small cell lung cancer growth and progression through sponging miR-5095. American Journal of Translational Research 11(2): 895–903.
16. Chen B and Ling CH (2019) Long noncoding RNA AK027294 acts as an oncogene in non-small cell lung cancer by up-regulating STAT3. European Review for Medical and Pharmacological Sciences 23(3): 1102–1107.
17. Wang X, Zhang G, Cheng Z, et al. (2018) Knockdown of LncRNA-XIST suppresses proliferation and TGF-β1-induced EMT in NSCLC through the Notch-1 pathway by regulation of miR-137. Genetic Testing and Molecular Biomarkers 22(6): 333–342.
18. Xu Z, Xu J, Lu H, et al. (2017) LARP1 is regulated by the XIST/miR-374a axis and functions as an oncogene in non-small cell lung carcinoma. Oncology Reports 38(6): 3659–3667.
19. Li C, Wan L, Liu Z, et al. (2018) Long non-coding RNA XIST promotes TGF-β-induced
epithelial-mesenchymal transition by regulating miR-367/141-ZEB2 axis in non-small-cell lung cancer. Cancer Letters 418: 185–195.

20. Jiang H, Zhang H, Hu X, et al. (2018) Knockdown of long non-coding RNA XIST inhibits cell viability and invasion by regulating miR-137/PXN axis in non-small cell lung cancer. International Journal of Biological Macromolecules 111:623–631.

21. XHan Q, Li L, Liang H, et al. (2017) Downregulation of lncRNA X inactive specific transcript (XIST) suppresses cell proliferation and enhances radiosensitivity by upregulating mir-29c in nasopharyngeal carcinoma cells. Medical Science Monitor 23: 4798–4807.

22. Valdmanis PN, Kim HK, Chu K, et al. (2018) miR-122 removal in the liver activates imprinted microRNAs and enables more effective microRNA-mediated gene repression. Nature Communications 9: 5321.

23. Tam C, Wong JH, Tsui SKW, et al. (2019) LncRNAs with miRNAs in regulation of gastric, liver, and colorectal cancers: Updates in recent years. Applied Microbiology and Biotechnology 103(12): 4649–4677.

24. Braicu C, Zimta AA, Haragus A, et al. (2019) The function of non-coding mas in lung cancer tumorigenesis. Cancers (Basel) 11(5): 605.

25. Tomasik B, Chalubińska-Fendler J, Chowdhury D, et al. (2018) Potential of serum microRNAs as biomarkers of radiation injury and tools for individualization of radiotherapy. Translational Research 201: 71–83.

26. Ni J, Bucci J, Chang L, et al. (2017) Targeting microRNAs in prostate cancer radiotherapy. Theranostics 7(13): 3243–3259.

27. El Bezawy R, Tinelli S, Tortoreto M, et al. (2019) miR-205 enhances radiation sensitivity of prostate cancer cells by impairing DNA damage repair through PKCe and ZEB1 inhibition. Journal of Experimental & Clinical Cancer Research 38(1): 51.

28. Wang F, Mao A, Tang J, et al. (2019) MicroRNA-16-5p enhances radiosensitivity through modulating Cyclin D1/E1-pRb-E2F1 pathway in prostate cancer cells. Journal of Cellular Physiology 234(8): 13182–13190.

29. Ghiasi N, Habibagahi M, Rosli R, et al. (2014) Tumour suppressive effects of WEE1 gene silencing in breast cancer cells. Asian Pacific Journal of Cancer Prevention 14(11): 6605–6611.

30. Do K, Doroshow JH and Kummar S (2013) Wee1 kinase as a target for cancer therapy. Cell Cycle 12(19): 3159–3164.

31. Kim HY, Cho Y, Kang H, et al. (2016) Targeting the WEE1 kinase as a molecular targeted therapy for gastric cancer. Oncotarget 7(31): 49902–49916.

32. Qi W, Xu X, Wang M, et al. (2019) Inhibition of Wee1 sensitizes AML cells to ATR inhibitor VE-822-induced DNA damage and apoptosis. Biochemical Pharmacology 164: 273–282.

33. Lee JW, Parameswaran J, Sandoval-Schaefer T, et al. (2019) Combined Aurora Kinase A (AURKA) and WEE1 inhibition demonstrates synergistic anti-tumor effect in squamous cell carcinoma of the head and neck. Clinical Cancer Research 25(11): 3430–3442.

34. Magnusson GI, Holm R, Emilsen E, et al. (2012) High expression of Wee1 is associated with poor disease-free survival in malignant melanoma: potential for targeted therapy. PLoS One 7(6): e38254.

35. PosthumaDeBoer J, Würdinger T, Graat HC, et al. (2011) WEE1 inhibition sensitizes osteosarcoma to radiotherapy. BMC Cancer 11: 156.

36. Karnak D, Engelke CG, Parsels LA, et al. (2014) Combined inhibition of Wee1 and PARP1/2 for radiosensitization in pancreatic cancer. Clinical Cancer Research 20(19): 5085–5096.

37. Parsels LA, Karnak D, Parsels JD, et al. (2018) PARP1 trapping and DNA replication stress enhance radiosensitization with combined WEE1 and PARP inhibitors. Molecular Cancer Research 16(2): 222–232.

38. Fan L, Qi H, Teng J, et al. (2016) Identification of serum miRNAs by nano-quantum dots microarray as diagnostic biomarkers for early detection of non-small cell lung cancer. Tumor Biology 37(6): 7777–7784.

39. Lee JH, Sung JY, Choi EK, et al. (2019) C/EBPβ is a transcriptional regulator of Wee1 at the G2/M phase of the cell cycle. Cells 8(2): 145.

40. Zhang H, Zhang DX, Ju T, et al. (2019) The effect of postoperative radiotherapy on the survival of patients with resectable stage III-N2 non-small-cell lung cancer: A systematic review and meta-analysis. Neoplasma 66(5): 717–726.

41. Li Q, Zong Y, Li K, et al. (2019) Involvement of endothelial CK2 in the radiation induced perivascular resistant niche (PVRN) and the induction of radiosensitization in A549 cells. Journal of Cellular Physiology 234(8): 13182–13190.

42. Xiong K, Shao LH, Zhang HQ, et al. (2018) MicroRNA-9 functions as a tumor suppressor and radiosensitization with combined WEE1 and PARP inhibitors. International Journal of Cancer 143(11): 2738–2744.

43. Magnussen GI, Holm R, Emilsen E, et al. (2012) High expression of Wee1 is associated with poor disease-free survival in malignant melanoma: potential for targeted therapy. PLoS One 7(6): e38254.
lung adenocarcinoma by interacting with HOXA10. *Journal of Cellular Physiology* 234(7): 11805–11821.

45. Chen J, Shen Z, Zheng Y, et al. (2015) Radiotherapy induced Lewis lung cancer cell apoptosis via inactivating β-catenin mediated by upregulated HOTAIR. *International Journal of Clinical and Experimental Pathology* 8(7):7878–7886.

46. Fang J, Sun CC and Gong C (2016) Long noncoding RNA XIST acts as an oncogene in non-small cell lung cancer by epigenetically repressing KLF2 expression. *Biochemical and Biophysical Research Communications* 478(2): 811–817.

47. Song P, Ye LF, Zhang C, et al. (2016) Long non-coding RNA XIST exerts oncogenic functions in human nasopharyngeal carcinoma by targeting miR-34a-5p. *Gene* 592(1): 8–14.

48. Li H, Jin X, Chen B, et al. (2018) Autophagy-regulating microRNAs: Potential targets for improving radiotherapy. *Journal of Cancer Research and Clinical Oncology* 144(9): 1623–1634.

49. Caiola E, Frapolli R, Tomanelli M, et al. (2018) Wee1 inhibitor MK1775 sensitizes KRAS mutated NSCLC cells to sorafenib. *Scientific Reports* 8(1): 948.

50. Yoshida T, Tanaka S, Mogi A, et al. (2004) The clinical significance of Cyclin B1 and Wee1 expression in non-small-cell lung cancer. *Annals of Oncology* 15(2): 252–256.

51. Ku BM, Bae YH, Koh J, et al. (2017) Mutational status of TP53 defines the efficacy of Wee1 inhibitor AZD1775 in KRAS-mutant non-small cell lung cancer. *Oncotarget* 8(40): 67526–67537.