Knockdown long non-coding RNA HCP5 enhances the radiosensitivity of esophageal carcinoma by modulating AKT signaling activation

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**ABSTRACT**

Recently, long noncoding RNAs (lncRNAs) have been revealed to participate in cancer therapy. Especially in tumor radiotherapy, lncRNAs usually could enhance or restrict the radiosensitivity in different ways. LncRNA HCP5 is highly expressed in esophageal cancer and influenced the malignant behaviors of esophageal cancer cells. However, this study dedicates to clarify if lncRNA HCP5 affects the radiosensitivity of esophageal carcinoma. The expression levels of HCP5 in esophageal cancer and adjacent noncancerous tissue were first analyzed on the TCGA database and then detected by qRT-PCR. The related functional experiments were used to investigate whether the radiosensitivity of esophageal squamous cell carcinoma was affected by the inhibition of HCP5. The expression results showed HCP5 is upregulated in esophageal cancers compared to the normal tissues. Meanwhile, knockdown HCP5 further suppressed the proliferation and promoted the apoptosis of esophageal cancer cells treated with a 2 Gy dose of radiotherapy. Moreover, we uncovered that knockdown HCP5 eliminated radiotherapy resistance by modulating the miR-216a-3p/PDK1 axis to inhibit the AKT activation. Finally, rescue experiments pointed that lowering the miR-216a-3p expression weakened the inhibition effect of knockdown HCP5 on cells treated with radiotherapy. To summarize, our results indicate that HCP5 is involved in esophageal carcinoma radiotherapy and knockdown HCP5 enhances the radiosensitivity of esophageal carcinoma by modulating AKT signaling activation.

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**Introduction**

Esophageal carcinoma (EC) endangers severely public health with a continually increasing incidence [1,2]. Statistically, esophageal cancer has become the most common and lethal cancer type in the world, ranking 8\textsuperscript{th} and 6\textsuperscript{th} in 2018, respectively [2,3]. Up to date, though esophagectomy with radical lymphadenectomy is preferred for esophageal cancer treatment, radiation, and chemotherapy have also been widely applied for patients with esophageal cancer [1]. Radiotherapy has emerged as an advantageous therapy alternative for EC patients who are medically inoperable to undergo surgery [1,4]. Whereas still a portion of EC patients cannot profit or poorly benefit from radiotherapy. Improving the radiotherapy sensitivity of tumors is very beneficial to the radiotherapy effect [4,5]. Thus, it is urgent to excavate the underlying mechanism of esophageal cancer radiotherapy for improving the radiosensitivity of esophageal cancer.

LncRNA HCP5 (HLA Complex P5) is a kind of RNA gene without protein-coding [6]. Studies have pointed that lncRNA HCP5 is associated with the progressions and developments of various cancers and diseases [6,7]. For instance, lncRNA HCP5 typically acts an oncogenic role to promote the malignant behaviors in thyroid cancer, colorectal cancer, and gastric cancer [8–10]. Moreover, in esophageal cancer, HCP5 induces PI3K/AKT/mTOR signaling to accelerate the development [7]. However, there are few studies that reported that HCP5 is connected...
with the radiosensitivity of cancer. Here in this study, we attempt to elucidate whether the radiosensitivity of esophageal cancer is governed by HCP5.

It is well known that IncRNA sever a ceRNA (Competing endogenous RNA) function to downregulate the specific miRNAs, which are capable of binding with IncRNA [11]. Through an online database analysis, miR-216a-3p is a downward target of HCP5 with high grades. Although a large number of researchers found that miR-216a-3p has tumor-suppressing function, no works have made the connection between the miR-216a-3p and radiotherapy [12,13]. Similarly, miRNAs could bind at 3’UTR of a target gene to inhibit its expression [11,14]. PDK1 (Pyruvate Dehydrogenase Kinase 1) mainly activates the PDH (Pyruvate dehydrogenase) enzyme, which catalyzes the oxidative decarboxylation of pyruvate [15,16]. Through a series of catalytic reactions and signaling transduction, the AKT signaling would be phosphorylated after inducing PDK1 expression [17]. Interestingly, the activation of AKT signaling is closely related to the radiosensitivity of esophageal carcinoma [18,19]. Regardless of the cancer type, the emerging consensus is that inhibition of AKT activation could elevate the radiotherapy curative effect in most cancers [20,21]. Thus, reducing the level of AKT phosphorylation is essential for improving the response to radiotherapy in esophageal cancer.

To sum up, we proposed that IncRNA HCP5 could participate in the radiotherapy of esophageal carcinoma. Knockdown of HCP5 modulates the miR-216a-3p/PDK1 axis to inhibit AKT activation, eventually improving the radiosensitivity of esophageal cancer. In all, our study provides a novel radiotherapy strategy or regimen for esophageal cancer. Our presumption would help more esophageal cancer patients benefit from radiotherapy.

**Methods**

**Patient tissues**

Total 20 pairs of esophageal cancer and adjacent noncancerous tissues were collected from 20 esophageal cancer patients between 2019 and 2020 with informed written consent. The tissues were immediately frozen in liquid nitrogen after collection and then transferred to a – 80°C refrigerator.

**Cell culture**

Esophageal cancer cells (EC9706, KYSE30, and TE-1) and Human esophageal epithelial cells (Het-1A) were all cultured in RPMI 1640 medium (Gibco, USA) with 10% fetal bovine serum (Gibco, USA) in a 37°C, 5% CO2 incubator. All cells were obtained from Shanghai Cell Bank of Type Culture Collection of Chinese Academy of Sciences.

**qRT-PCR**

The qRT-PCR was used to detected the HCP5, miR-216a-3p, and PDK1 expression in tissues and cell lines. The RNA of tissues and cells were extracted by Trizol (Invitrogen, Carlsbad, CA, USA) and then reversed to cDNA with a reverse transcription kit (Invitrogen). GAPDH and U6 were, respectively, as internal controls for mRNA and miRNA. The analysis was performed with SYBR (TaKaRa, China) on a CFX96 RT-PCR system. The primers in this study are shown in Table 1.

**Cell transfection**

Vectors encoding short-hairpin HCP5 (shHCP5) and the negative control (sh-control) were designed and synthesized by Genechem (Genechem Shanghai, China). The corresponding sequence of shHCP5 is 5’-GCTGGTCTCTGGACACATACTCTCGAGAGTGGTGTCGTGGAGTCG-3’. The KYSE30 and TE-1 cells were infected with the lentivirus shRNA HCP5 and corresponding negative control (shHCP5 and shNC) to knockdown of HCP5 (MOI = 50). The miR-216a-3p mimic, miR NC, miR-216a-3p inhibitor and inhibitor NC were all synthesized to interfere the miR-216a-3p level.

| Name   | Forward/Reverse | Sequence (5’ to 3’) |
|--------|-----------------|---------------------|
| HCP5   | F               | CCGCTGTCGTCGGACACATACTCTCGAGAGTGGTGTCGTGGAGTCG |
|        | R               | CTACCGTCGTCGGATTTTGC |
| miR-216a-3p | F       | TATCTCAGCTGGCAACTCTCGAGAGTGGTGTCGTGGAGTCG |
|        | R               | GGTGTGTCGGAGTGGTGTCGTGGAGTCG |
| PDK1   | F               | CTGTTGATACGGATCAGAAGAACC |
|        | R               | TCCACAAACATAAAGAGATGCT |
| GAPDH  | F               | GAAGGTGAAGGTCGGAGTGCGAGAAGGTCGGAGTGCGAGAAGGTCGGAGTGCGAG |
|        | R               | GAAGGTGAAGGTCGGAGTGCGAGAAGGTCGGAGTGCGAGAAGGTCGGAGTGCGAG |
| U6     | F               | CTGGTTGACGCAAGAAC |
|        | R               | AACGCTCAGAATTTGGCT |
(RiBoBio, Guangzhou, China). About at 60% confluence, cells were treated with Lipofectamine™2000 (ThermoFisher, USA) and required miR mimic, miR inhibitor or NC.

**Cell viability assays**

3000 cells were planted into a 96-well plate for 24 h. And then according to the group, cells were exposed to 0 or 2 Gy radiation (once). After 3 days, the cell viability was evaluated by adding 10 μl CCK-8 (Beyotime, Shanghai, China) for another 4 h. The results are read on 450 nm by a Microplate Reader.

**Colony assays**

About 500 cells were planted into a six-well plate for 2 days. And then according to the group, cells were exposed to a range of radiation doses (0, 2, 4, 6, and 8 Gy) (once). After 10 days, the cell colon was stained and counted. More than 50 cells were identified as a colony.

**Apoptosis**

The corresponding cells were harvested at 72 h post-transfection. A single dose of radiotherapy was given 1 day after transfection. The apoptosis cells were examined by an Annexin V-FITC/PI kit (Invitrogen, USA) on flow cytometry (Beckman Coulter).

**Luciferase reporter system**

A luciferase reporter vectors (pRL-TK, Promega) were used to detect the luciferase intensity. The corresponding plasmids with the wild-type sequence or mutant sequence were synthesized from GenePharma. After 48 h at co-transfection with luciferase plasmids and miR mimic or NC, the intensity was detected by Dual-Luciferase Reporter Assay System (Promega).

**Western blotting**

The cells are lysed by RIPA Lysis Buffer (Beyotime, Shanghai, China) to get protein, and then centrifugation and degeneration. The samples were separated by 10% SDS-PAGE gels and transferred to PVDF membrane (Bio-Rad, USA). The primary antibody, anti-PDK1 (1:1000, ab202468, abcam), p-AKT (1:1000, ab38449, abcam), t-AKT (1:1000, ab8805, abcam) and GAPDH (1:5000, ab8245, abcam) was incubated after blocking with blocking buffer overnight. On the second day, HRP conjugated secondary antibodies were incubated after washing. The results were examined by chemiluminescence. Anti-GAPDH was used as internal reference.

**Statistical analysis**

Statistical analysis was performed using SPSS 19.0 software (SPSS, Chicago, IL, USA). The data are expressed as the mean ± standard deviation (SD). Differences between groups were evaluated by Student’s t-test. P < 0.05 indicated statistical significance.

**Results**

Radiotherapy is a common and effective treatment for cancer. This study sets out to explore whether knockdown HCP5 enhances the efficacy of radiotherapy for esophageal carcinoma. Due to HCP5 is upregulated in esophageal cancer, we constructed a lentiviral shRNA to knockdown HCP5. Through a series of functional experiments, we revealed that knockdown IncRNA HCP5 enhances the radiosensitivity of esophageal carcinoma by modulating AKT signaling activation.

**The higher expression of IncRNA HCP5 is in esophageal cancer tissues**

Through analyzing the expression profile of HCP5 in esophageal cancer tissues and adjacent tissues on an online database (http://gepia.cancer-pku.cn), we found that HCP5 is upregulated in esophageal cancer tissues compared with normal tissues (Figure 1(a)). Similarly in our collected samples (Figure 1(b)), the expression level of HCP5 in esophageal tissues is higher than adjacent tumor tissues. Meanwhile, we detected the expression in esophageal cancer cells (EC9706, KYSE30, and TE-1) and normal esophageal cells (Het-1A). The result shows that HCP5 arises in all three cancer
cells compared to the Het-1A cell (Figure 1(c)). In addition, after a single dose of radiation, the HCP5 level in KYSE30 and TE-1 cells are apparently elevated with the increase in time or dose (Figure 1(d,e)). All the expression profile results indicated that HCP5 is upregulated in esophageal cancer and is also lifted by radiation.

**Knockdown of HCP5 further inhibits esophageal cancer cells proliferation and promotes apoptosis combined with a single dose of radiation**

We constructed a lentiviral shRNA to knockdown HCP5 expression in KYSE30 and TE-1 cells. As shown in Figure 2(a), the HCP5 level is obviously repressed after infecting with the lentiviral shRNA. Subsequently, we divided the cells for four groups (1. shNC + 0 Gy, 2. shHCP5 + 0 Gy, 3. shNC + 2 Gy, 4. shHCP5 + 2 Gy) to investigate whether knockdown of HCP5 could further inhibit the malignant behaviors of esophageal cancer cells combined with a single dose of radiation. ShNC or shHCP5 esophageal cancer cells were treated with a single 2 Gy dose of radiation on day 0. After 3 days, the CCK-8 assays showed that knockdown of HCP5 or 2 Gy radiation alone both suppressed the cell viability on KYSE30 and TE-1 cells, however, HCP5 knockdown plus 2 Gy radiation exhibited the strongest inhibitory effect compared to the other 3 groups (Figure 2(b)). Similarly, in Figure 2(c), it also reflected consistent results that the clonal proliferation of cells was the weakest in the combination therapy group. Moreover, Knockdown of HCP5 further promotes KYSE30 and TE-1 cell apoptosis combined with a single 2 Gy dose of radiation (Figure 2(d)). From the results of these trials, we can consider that HCP5 is associated with esophageal cancer radiotherapy.

**Knockdown HCP5 enhances the efficacy of radiotherapy for esophageal carcinoma**

To clearly clarify whether knockdown HCP5 enhances the efficacy of radiotherapy for esophageal carcinoma, a single-hit, multi-target model that commonly and standardly evaluates the experimental irradiation fractionation was to apply. According to the model requirements, shNC or shHCP5 KYSE30 and TE-1 cells were exposed to the different radiotherapy doses...
As shown in Figure 3(b,d), the cellular survival curves of two esophageal cancer cells pointed that HCP5 repressed indeed elevates the radiosensitivity of esophageal carcinoma. The relative radiosensitization effects data (such as D0, Dq, N, and SF2) confirmed this sensibilization as well in Table 2. Thus, we can conclude that knockdown HCP5 enhances the efficacy of radiotherapy for esophageal carcinoma.
Figure 3. Knockdown HCP5 enhances the efficacy of radiotherapy for esophageal carcinoma. (a) and (c), The colony formation was examined in KYSE30 and TE-1 cells with different dose of radiation; (b) and (d), The cellular survival curves of KYSE30 and TE-1 cells.

Table 2. The relative radiosensitization effects in KYSE30 and TE-1.

|          | D0 (Gy)  | Dq (Gy)  | N      | SF2 (%) |
|----------|----------|----------|--------|---------|
| KYSE30   |          |          |        |         |
| shNC     | 2.957 ± 0.113 | 1.881 ± 0.053 | 1.889 ± 0.024 | 73.9 ± 0.2 |<|0.05 |
| shHCP5   | 1.740 ± 0.102* | 1.489 ± 0.047 | 1.354 ± 0.031* | 59.2 ± 0.1* |
| TE-1     |          |          |        |         |
| shNC     | 3.004 ± 0.149 | 0.751 ± 0.042 | 1.216 ± 0.062 | 59.6 ± 0.2 |
| shHCP5   | 1.853 ± 0.124* | 0.901 ± 0.113 | 1.688 ± 0.109* | 52.1 ± 0.1* |

Unpaired student t test. * P < 0.05.

HCP5 functions by regulating miR-216a-3p/PDK1 axis

As to how HCP5 functions in esophageal carcinoma radiotherapy, we speculate that IncRNA HCP5 should sever a ceRNA participating in regulation. By analyzing an online database (http://starbase.sysu.edu.cn/), the latent binding site between HCP5 and miR-216a-3p is gained in Figure 4(a). Interestingly, we also found that the miR-216a-3p occurred a significant augment after KYSE30 and TE-1 cells knockdown of HCP5 (Figure 4(b)). Then, the miR-216a-3p mimic was synthesized to verify this interaction (Figure 4(c)). As shown in Figure 4(d), miR-216a-3p only affected the fluorescence intensity of wild-type HCP5 reporter but did not affect that of mutant-type. Also analyzing the target of miR-216a-3p, it indicates that miR-216a-3p could bind the 3’UTR of PDK1 (Figure 4(e)). The luciferase assays also confirmed that miR-216a-3p interacts with the 3’UTR of PDK1 (Figure 4(f)). Moreover, miR-216a-3p mimic both inhibits the expression of PDK1 on mRNA and protein level (Figure 4(g, h)). Therefore, HCP5 should be supposed to functions by regulating miR-216a-3p/PDK1 axis.

Knockdown HCP5 modulates the miR-216a-3p/PDK1 axis to inhibit AKT activation

To prove HCP5 regulates the radiosensitivity of esophageal carcinoma by miR-216a-3p/PDK1 axis, the miR-216a-3p inhibitor was performed in rescue experiments (Figure 5(a)). Before the rescue experiments, we confirmed that knockdown HCP5 directly inhibits the protein expression of PDK1 (Figure 5(b)). Subsequently, all experiments were treated with a single 2 Gy dose of radiation. As shown in Figure 5(c), miR-216a-3p inhibitor weakened the inhibition effect of knockdown HCP5 on cell viability. In Figure 5(d), the colony numbers of shHCP5 with
miR-216a-3p inhibitor group picked up compared to shHCP5 with inhibitor NC. Meanwhile, the previously high rate of shHCP5 cells apoptosis was also suppressed by miR-216a-3p inhibitor compared to inhibitor NC (Figure 5(e)). From the molecular point of view, these results should be attributed to the change of the AKT signaling activation (Figure 5(f)). Based on these results, we concluded that knockdown HCP5 modulates the miR-216a-3p/PDK1 axis to inhibit AKT activation.

Discussion

Our study first proposed that lncRNA HCP5 is associated with the radiosensitivity of esophageal carcinoma and affected the esophageal cancer radiotherapy curative effect. Mechanistically, HCP5 restraints miR-216a-3p expression and miR-216a-3p inhibits the expression of PDK1. Furthermore, knockdown of HCP5 decreased the p-AKT level by miR-216a-3p/PDK1 axis to increase the radiosensitivity of esophageal cancer cells. In addition, the rescue experiments also indicated miR-216a-3p inhibitor weakened the inhibition effect of knockdown HCP5 on cells treated with radiotherapy. In all, our results concluded that HCP5 is involved in esophageal carcinoma radiotherapy and knockdown HCP5 enhances the radiosensitivity of esophageal carcinoma by modulating AKT signaling activation.

Esophagectomy with radical lymphadenectomy is preferred for esophageal cancer treatment, notwithstanding, still a number of patients medically are inoperable to undergo surgery [1,22]. Due to the complication of dysphagia occurs with high-frequency in the advanced esophageal cancer
patients, radiotherapy is the most appropriate for these patients [1,23]. It is beneficial for patients with esophageal cancer that enhance the radiosensitivity of esophageal cancer [23,24]. Therefore, excavating a novel target for elevating the radiotherapy response to esophageal cancer is imminent.

To date, there are rare studies linked the HCP5 with cancer therapy. Based on our results, we first revealed that lncRNA HCP5 definitely participates in the response to radiotherapy for esophageal cancer. A series of radiosensitization effects data (such as D0, Dq, N, and SF2) give a shred of strong evidence that knockdown of HCP5

**Figure 5.** Knockdown HCP5 modulates the miR-216a-3p/PDK1 axis to inhibit AKT activation. (a), The expression of miR-216a-3p in KYSE30 and TE-1 cells with miR-216a-3p inhibitor; (b), The protein expression of PDK1 in shHCP5 KYSE30 and TE-1 cells; (c), The cell viability was detected by CCK-8 assay in KYSE30 and TE-1 cells; (d), The colony formation was examined in KYSE30 and TE-1 cells; (e), The apoptosis was examined in KYSE30 and TE-1 cells; (f), The activation AKT signaling in KYSE30 and TE-1 cells was examined by Western blot. Data are presented as mean ± SD, n = 5. * compared to the group of shNC + inhibitor NC; *P < 0.05, **P < 0.01; # compared to other group of shHCP5 + inhibitor NC; #P < 0.05.
enhances the radiosensitivity of esophageal cancer. Moreover, it is showed that the AKT signaling, which closely associating with cancer radiotherapy, is affected by HCP5. For example, Wang et al. reported that IncRNA TUG1 could regulate the miR-144-3p/MET axis to induce the AKT activation, eventually rise a radiation resistance effect on esophageal cancer [25]. Other studies also indicated that no matter what the genes or IncRNAs involved in AKT signaling activation could influence cancer radiotherapy response [26–28]. Hence, our results are consistent with the common strategy of radiotherapy sensitization. In sum, IncRNA HCP5 is a key regulator of radiotherapy response in esophageal cancer.

However, our work only focuses on the role of HCP5 in esophageal radiotherapy. The other cancer treatments, such as chemotherapy and immunotherapy, should be also affected by HCP5 repressed. It is generally known that AKT signaling is closely associated with chemotherapy and immunotherapy [29–31]. Once knockdown of IncRNA HCP5 reduced the phosphorylation level of AKT, the therapeutic effect evaluation of chemotherapy and immunotherapy usually get the difference. Meanwhile, the radiosensitivity of esophageal cancer involved pathway includes DNA damage and repair should also be investigated. The DNA damage level is a key factor in the radiosensitivity of esophageal cancer [32]. This theoretical knowledge inspired us to further investigate the cross-link between HCP5 and cancer treatments.

It is possible that HCP5 may regulate the radiotherapy response to esophageal cancer in another way. In addition, the role of miR-216a-3p or PDK1 alone in esophageal cancer radiotherapy response is needed to conduct a study. However, our study has given a complete line of evidence supporting the knockdown of HCP5 enhance esophageal cancer radiosensitivity. Even so, other mechanisms should be also discussed in our next work.

**Conclusion**

In summary, our results indicate that HCP5 is involved in esophageal carcinoma radiotherapy and knockdown HCP5 enhances the radiosensitivity of esophageal carcinoma by modulating AKT signaling activation.

**Abbreviations**

EC: esophageal carcinoma; IncRNAs: long non-coding RNAs; ceRNA: competing endogenous RNA; HCP5: HLA Complex P5; miRNAs: MicroRNAs; PDK1: pyruvate dehydrogenase kinase 1; PDH: pyruvate dehydrogenase

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**Ethics and consent statement**

This study was performed with the approval of the Research Ethics Committee of Hubei University of Medicine. In addition, written informed consent forms were signed by all the patients who participated in this research.

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**References**

[1] Yang YM, Hong P, Xu WW, et al. Advances in targeted therapy for esophageal cancer. Signal Transduct Target Ther. 2020;5(1). DOI:10.1038/s41392-020-00323-3.

[2] Mattiuzzi C, Lippi G. Current cancer epidemiology. J Epidemiol Glob Health. 2019;9(4):217–222.

[3] Ferlay J, Colombet M, Soerjomataram I, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer. 2019;144(8):1941–1953.

[4] Su M, Xiao YH, Ma JL, et al. Long non-coding RNAs in esophageal cancer: molecular mechanisms, functions, and potential applications. J Hematol Oncol. 2018 Sep 17;11. doi:10.1186/s13045-018-0663-8.

[5] Coleman HG, Xie SH, Lagergren J. The epidemiology of esophageal adenocarcinoma. Gastroenterology. 2018;154(2):390–405.

[6] Qin SY, Yang L, Kong S, et al. IncRNA HCP5: a potential biomarker for diagnosing gastric cancer. Front Oncol. 2021;11. DOI:10.3389/fonc.2021.684531.
[7] Zou SB, Gao Y, Zhang ST. lncRNA HCP5 acts as a ceRNA to regulate EZH2 by sponging miR-138-5p in cutaneous squamous cell carcinoma. Int J Oncol. 2021;59(2):56.

[8] Chen J, Zhao DJ, Meng Q. Knockdown of HCP5 exerts tumor-suppressive functions by up-regulating tumor suppressor miR-128-3p in anaplastic thyroid cancer. Biomed Pharmacother. 2019;116:108966.

[9] Bai N, Ma Y, Zhao J, et al. Knockdown of lncRNA HCP5 suppresses the progression of colorectal cancer by miR-299-3p/PFN1/AKT Axis. Cancer Manag Res. 2020;12:4747–4758.

[10] Gao M, Liu L, Yang Y, et al. LncRNA HCP5 induces gastric cancer cell proliferation, invasion, and EMT processes through the miR-186-5p/WNT5A axis under hypoxia. Front Cell Dev Biol. 2021;9. DOI:10.3389/fcell.2021.663654.

[11] Karreth FA, Pandolfi PP. ceRNA cross-talk in cancer: when ce-bling rivals go awry. Cancer Discov. 2013;3(10):1113–1121.

[12] Zhao J, Li L, Yang T. MiR-216a-3p suppresses the proliferation and invasion of cervical cancer through downregulation of ACTL6A-mediated YAP signaling. J Cell Physiol. 2020;235(12):9718–9728.

[13] Wu YF, Zhang J, Zheng Y, et al. miR-216a-3p inhibits the proliferation, migration, and invasion of human gastric cancer cells via targeting RUNX1 and activating the NF-kappa B signaling pathway. Oncol Res. 2018;26(1):157–171.

[14] Ning ML, Qin S, Tian J, et al. LncRNA AFAP-AS1 promotes anaplastic thyroid cancer progression by sponging miR-155-5p through ETS1/ERK pathway. Bioengineered. 2021;12(1):1543–1554.

[15] Peters SJ. Regulation of PDH activity and isoform expression: diet and exercise. Biochem Soc Trans. 2003;31:1274–1280.

[16] Zhang W, Hu X, Chakravarty H, et al. Identification of novel pyruvate dehydrogenase kinase 1 (PDK1) inhibitors by kinase activity-based high-throughput screening for anticancer therapeutics. ACS Comb Sci. 2018;20(11):660–671.

[17] Molinaro C, Martorati A, Lescuyer A, et al. 3-phosphoinositide-dependent protein kinase 1 (PDK1) mediates crosstalk between Src and Akt pathways in MET receptor signaling. FEBS Lett. 2021;595(21):2655–2664.

[18] Wang C, Li S, Liu J, et al. Silencing of S-phase kinase-associated protein 2 enhances radiosensitivity of esophageal cancer cells through inhibition of PI3K/AKT signaling pathway. Genomics. 2020;112(5):3504–3510.

[19] Zhang Y, Chen W, Wang H, et al. Upregulation of miR-519 enhances radiosensitivity of esophageal squamous cell carcinoma trough targeting PI3K/AKT/mTOR signaling pathway. Cancer Chemother Pharmacol. 2019;84(6):1209–1218.

[20] Chang I, Graham PH, Ni J, et al. Targeting PI3K/Akt/mTOR signaling pathway in the treatment of prostate cancer radioresistance. Crit Rev Oncol Hematol. 2015;96(3):507–517.

[21] Mardanshahi A, Gharibkandi NA, Vaseghi S, et al. The PI3K/AKT/mTOR signaling pathway inhibitors enhance radiosensitivity in cancer cell lines. Mol Biol Rep. 2021;48(8):6167–6180.

[22] Zhang WX, Yu DL, Peng JH, et al. Gastric-tube versus whole-stomach esophagectomy for esophageal cancer: a systematic review and meta-analysis. Plos One. 2017;12(3):e0173416.

[23] Zhang HF, Si J, Yue J, et al. The mechanisms and reversal strategies of tumor radioresistance in esophageal squamous cell carcinoma. J Cancer Res Clin Oncol. 2021;147(5):1275–1286.

[24] Demarest CT, Chang AC. The landmark series: multitarget therapy for esophageal cancer. Ann Surg Oncol. 2021;28(6):3375–3382.

[25] Wang P, Yang ZB, Ye T, et al. lncTUG1/miR-144-3p affect the radiosensitivity of esophageal squamous cell carcinoma by competitively regulating c-MET. J Exp Clin Cancer Res. 2020;39(1):7.

[26] Cui Z, Liu J, Sun Q, et al. GPR120 promotes radiation resistance in esophageal cancer via regulating AKT and apoptosis pathway. Anticancer Drugs. 2021;32(1):53–60.

[27] Zhang FQ, Shao C, Chen Z, et al. Low dose of trichosatin A improves radiation resistance by activating Akt/Nrf2-dependent antioxidation pathway in cancer cells. Radiat Res. 2021;195(4):366–377.

[28] Tang Y, Liu B, Li J, et al. Genetic variants in PI3K/AKT pathway are associated with severe radiation pneumonitis in lung cancer patients treated with radiation therapy. Cancer Med. 2016;5(1):24–32.

[29] Kaboli PJ, Salimian F, Aghapour S, et al. Akt-targeted therapy as a promising strategy to overcome drug resistance in breast cancer - A comprehensive review from chemotheroapy to immunotherapy. Pharmacol Res. 2020;156:104806.

[30] Estruch M, Reckzeh K, Vittori C, et al. Targeted inhibition of cooperative mutation- and therapy-induced AKT activation in AML effectively enhances response to chemotherapy. Leukemia. 2021;35(7):2030–2042.

[31] Han S, Wang P-F, Cai H-Q, et al. Alterations in the RTK/Ras/PI3K/AKT pathway serve as potential biomarkers for immunotherapy outcome of diffuse gliomas. Aging-Us. 2021;13(11):15444–15458.

[32] Wu LZ, Huang M-L, Qi C-L, et al. Overexpression of Notch2 enhances radiosensitivity via inhibition of the AKT/mTOR signaling pathway in nasopharyngeal carcinoma. Bioengineered. 2021;12(1):3398–3409.