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PII: S0171-9335(17)30309-6
DOI: https://doi.org/10.1016/j.ejcb.2018.04.005
Reference: EJCB 51009

To appear in:

Received date: 2-12-2017
Revised date: 30-3-2018
Accepted date: 10-4-2018

Please cite this article as: Zou Y, Yao S, Chen X, Liu D, Wang J, Yuan X, Rao J, Xiong H, Yu S, Yuan X, Zhu F, Hu G, Wang Y, Xiong H, LncRNA OIP5-AS1 regulates radioresistance by targeting *DYRK1A* through miR-369-3p in colorectal cancer cells, *European Journal of Cell Biology* (2010), https://doi.org/10.1016/j.ejcb.2018.04.005

LncRNA OIP5-AS1 regulates radioresistance by targeting *DYRK1A* through miR-369-3p in colorectal cancer cells

Running title: LncRNA OIP5-AS1 regulates radioresistance of colorectal cancer

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Abstract:

Object: This study aimed to investigate the role of lncRNA OIP5-AS1 in regulating radioresistance of colorectal cancer (CRC) cells.

Methods: Microarray analysis was used to screen out lncRNAs differentially expressed in radio-resistant CRC cell lines. Expression levels of OIP5-AS1, miR-369-3p and *DYRK1A* in CRC cell lines were measured by qRT-PCR. Protein expression of *DYRK1A* was determined by western blot. The target relationships among OIP5-AS1, miR-369-3p and *DYRK1A* were validated by dual luciferase reporter assay. Impacts of OIP5-AS1 or *DYRK1A* on CRC cellular activity and apoptosis were investigated by MTT assay, clonogenic survival assay and flow cytometry to analyze OIP5-AS1 or *DYRK1A*'s effect on radioresistance of CRC cells.

Results: LncRNA OIP5-AS1 and *DYRK1A* were down-regulated in radio-resistant CRC cell lines. OIP5-AS1 suppressed the expression of miR-369-3p, thus up-regulating *DYRK1A*, the downstream gene of miR-369-3p. OIP5-AS1 and *DYRK1A* impaired cell clonogenic survival and promoted cell apoptosis after irradiation, improving radiosensitivity of CRC cells.

Conclusion: LncRNA OIP5-AS1 suppressed cell viability, promoted radio-induced apoptosis, and enhanced the radiosensitivity of CRC cells by regulating *DYRK1A* expression through miR-369-3p.

Key words: colorectal cancer, OIP5-AS1, miR-369-3p, *DYRK1A*

Introduction

Colorectal cancer (CRC) is a malignant lesion of the colorectal mucosal epithelium caused by multiple factors including environmental, genetic and epigenetic ones (Luo et al., 2017). It is one of the most common cancers worldwide with the 5-year relative survival rates of about 60% (Siegel et al., 2017). Radiotherapy is widely used in CRC treatment to decrease local relapse and improve

survival (Lacombe et al., 2013). However, many patients suffer from radioresistance, which is a major obstacle of radiotherapy. Therefore, understanding the mechanisms of CRC and developing novel strategies to overcome radioresistance are of crucial importance.

Long noncoding RNAs (lncRNAs), which have more than 200 nucleotides (Nagano and Fraser, 2011), are reported to be involved in CRC development and be able to influence the chemo and radio resistance of CRC cells (Fanale et al., 2016; Luo et al., 2017). For instance, lncRNA HOTAIR contributed to radioresistance and aggravated the severity of CRC (Yang et al., 2016). LncRNA OIP5-AS1, the lncRNA transcribed in the antisense (AS) direction from the same gene that encodes Opa-interacting protein 5 (OIP5) (Kim et al., 2017), was initially identified as cyrano in zebrafish (Ulitsky et al., 2011). Its association with human malignancies has been identified in recent decades. For example, it reduced cell proliferation in cervical carcinoma by acting as a competing endogenous RNA (Kim et al., 2016), and facilitated apoptosis of multiple myeloma cells by modulating miR-410 (Yang et al., 2017). As reported by Deng *et al.*, OIP5-AS1 modulated the expression of Bcl-2 by targeting miR-448 in lung adenocarcinoma cells (Deng et al., 2018). However, up to now, the functions of lncRNA OIP5-AS1 in CRC have not been identified yet.

LncRNA affected CRC through interference with miRNAs, the small noncoding RNAs with about 21 nucleotides (Krol et al., 2010). By regulating target genes, miRNAs can sever as promotor or suppressor in tumor development. Previous studies suggested that miR-369 increased chemoresistance in non-small cell lung cancer (Hao et al., 2017) and induced cellular reprogramming in CRC (Ogawa et al., 2015). Nonetheless, the role of miR-369-3p played in the progress of CRC was not well understood, as well as its potential interaction with lncRNAs.

The dual-specificity tyrosine phosphorylation-regulated kinase-1A (*DYRK1A*) is a protein kinase member of the *DYRK* family, which includes five kinases such as *DYRK1B* and *DYRK2* (Duchon

and Herault, 2016). *DYRK1A* was reported to be functioned as either tumor-suppressor or oncogenic factor in different cancers (Abbassi et al., 2015; Fernandez-Martinez et al., 2015). It inhibited proliferation and reduced chemoresistance of acute myeloid leukemia cells (Liu et al., 2014). However, according to Jang *et al.*, *DYRK1A* promoted megakaryoblastic leukemia by inducing overexpression of cytokines (Jang et al., 2014). In CRC, *DYRK2* exerted suppressive effect on tumor development by restraining cell migration and invasion (Ito et al., 2017; Wang et al., 2017). *DYRK1A* might also be able to improve CRC deterioration, which needs further studies.

In consequence, our study attempted to investigate the effects of lncRNA OIP5-AS1 in CRC and reveal the mechanism related to miR-369-3p and *DYRK1A*. We found that lncRNA OIP5-AS1 could regulate radioresistance of CRC cells through miR-369-3p and *DYRK1A*. The results uncovered the interactions between lncRNA and miRNA in CRC and proposed novel targets for improving radiosensitivity of CRC cells.

Materials and Methods

Cell lines and culture

CRC cell lines LoVo and SW480 and human embryonic kidney cell line HEK293T were purchased from BeNa Culture Collection (BNCC, Suzhou, China). LoVo, SW480 and HEK293T cells were respectively preserved in 10% fetal bovine serum (FBS) F12K medium containing 2.5 g/L $NaHCO_3$ (Sigma-Aldrich, St. Louis, MO, USA), 10% FBS RPMI-1640 medium (GIBCO BRL, Grand Island, NY, USA) and 10% FBS DMEM medium (GIBCO BRL) at 37 ^oC and 5% CO_2 atmosphere.

Irradiation

Cells were x-ray irradiated at a dose rate of 200 cGy per minute at room temperature to a dose of 2 Gy each day, and incubated in the incubator after being irradiated with a total dose of 40 Gy. Radio-resistant cells were selected and named LoVo-RA and SW480-RA.

Microarray analysis

Differentially expressed lncRNAs associated with radiotherapy-resistance in colorectal cancer were screened through analyzing microarray dataset (Accession number: GSE95606) at the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/). In order to screen out differentially expressed lncRNAs, microarray data in GPL23146 platform were used to analyze cell lines LoVo and SW480 with or without exposure to 2 Gy dose of radiation. Differentially expressed lncRNAs were identified based on the criteria of fold change > 2 and $P < 0.05$.

QRT-PCR

Extracted by TRIzol® reagent (Invitrogen, Carlsbad, CA, USA) and PureLink RNA Mini Kit (Thermo Fisher Scientific, Waltham, MA, USA), total RNA in cells was reversely transcripted into cDNA by TIANScript II RT Kit (Tiangen, Beijing, China) according to manufacturer's instructions. Quantitative real-time PCR was subsequently performed with RealMasterMix SYBR Green Kit (Tiangen) and ABI7500 Applied Biosystems. Relative levels were normalized to GAPDH and calculated using $2^{-\Delta\Delta CT}$ method. PCR primers were obtained from Sangon Biotech (Shanghai, China) and listed in Table 1.

Cell transfection

OIP5-AS1 siRNA, miR-369-3p mimics, miR-NC mimics, NC siRNA, and OIP5-AS1 were purchased from Gene Pharma (Shanghai, China). The vector OIP5-AS1-pcDNA3.1 was constructed via subcloning OIP5-AS1 into pcDNA3.1 (Thermo Fisher Scientific). Cells were firstly planted in 6-well plates to culture for 24 h, then transfected by using Lipofectamine 3000 (Invitrogen) under instructions, and finally collected after 48 h.

Western blot

Total protein of cells was extracted using RIPA lysis buffer (Solarbio, Shanghai, China). After SDS-PAGE, proteins were transferred to 0.22 μm polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). The membrane was blocked with Bovine Serum Albumin (BSA) for 1 h and then incubated with the primary antibody Rabbit Anti-*DYRK1A* (1:1000, ab8418, Abcam, Cambridge, MA, USA), Mouse Anti-p53 antibody (1:500, ab1101, Abcam, Cambridge, MA, USA),

Rabbit Anti-p53 (phospho S15) (1:500, ab1431), Rabbit Anti-Caspase-9 (1:1000, ab202068), Rabbit Anti-Caspase-3 (1:1000, ab184787) and Rabbit Anti-GAPDH (1:2500, ab9485) at 4 ^{o}C overnight. Subsequently, the membrane was washed by TBST (TBS, 1 ml/L Tween-20) and incubated with the secondary antibody (HRP-labeled Anti-rabbit IgG, 1:1000, Cell Signaling Technology) at room temperature for 2 h. ECL Western Blotting Detection Kit from GE Healthcare (Amersham, UK) was applied to visualize the protein bands, which were then quantified by software Image Lab (Bio-Rad, Hercules, CA, USA).

Clonogenic survival assay

A predetermined number of cells (300 cells for 0 and 2 Gy, 500 cell for 4 Gy, and 3000 cells for 6 Gy) were seeded in 6-well plates. After being incubated for 24 h, the cells were irradiated with different doses of x-ray radiation (0, 2, 4, and 6 Gy) and then stored in an incubator for 10 to 14 days till the proper size of colonies were observed. The plates were then washed with PBS and each well was fixed with 2 ml methanol for 30 mins. Subsequently, cells were stained with Giemsa stain (Abcam) overnight. After washing and drying the plates in the next day, colonies with the cell number > 50 were counted.

MTT assay

Before MTT assay, cells were incubated for 2 days in the 96-well plates, with about 3000 cells (100 μl) per well. Then each well was injected with 10 μl MTT solution at the concentration of 5 mg/ml (pH=7.4) to incubate the cells for 4 h. After discarding the solution, 100 μl DMSO was added to each well to dissolve fonnazan crystals. Plates were shaken for 10 min to ensure adequate fonnazan solubility. The absorbance value at 490 nm was measured by microplate reader.

Cell apoptosis detection

Cells in 6-well plates were divided into two groups, i.e. 0 Gy group and IR group, containing LoVo, LoVo+si-OIP5-AS1, LoVo-RA, and LoVo-RA+ OIP5-AS. Transfected cells in IR group were irradiated with a dose of 6 Gy and cultured for 24 h. For apoptosis detection, cells were firstly digested by trypsin and re-suspended with binding buffer, then collected (100 μl cell suspension

with the concentration of 1×10^6 cells/ml) for 15-min staining, and finally detected by flow cytometer FACS Calibur (Becton Dickinson, San Jose, CA, USA). Cell staining was performed by 5 μl FITC Annexin V Apoptosis Detection Kits and PI from Becton Dickinson according to the instructions. Data were analyzed by FACS Diva (Becton Dickinson).

Dual luciferase reporter assay

The OIP5-AS1 cDNA with miR-369-3p wild-type binding sequence and OIP5-AS1 cDNA with miR-369-3p mutant binding sequence were cloned into luciferase reporter gene psiCHECK2, and named OIP5-AS1-WT and OIP5-AS1-Mut respectively. Similarly, vectors containing *DYRK1A* 3′-UTR with miR-369-3p wild-type binding sequence (*DYRK1A*-UTR-WT) or *DYRK1A* 3′-UTR with miR-369-3p mutant binding sequence (*DYRK1A*-UTR-Mut) were synthesized. WT vectors, Mut vectors, or empty reporter vector were respectively co-transfected with miR-369-3p or miR-NC into HEK293T cells. After being co-transfected for 48 h, cells were collected to measure the luciferase activities using Dual Luciferase Reporter Assay kit (Promega, Shanghai, China).

Statistical analysis

Statistics were presented as the mean ± SD. Comparison between two groups was performed with *t*-test and one-way ANOVA was used in case of multiple groups. All experiments were repeated in triplicate, and the data were analyzed by GraphPad Prism 6.0 (GraphPad Inc., La Jolla, CA, USA). $P < 0.05$ was considered as statistically significant.

Results

OIP5-AS1 and *DYRK1A* were down-regulated in radio-resistant CRC cell lines

According to microarray analysis, 33 differently expressed lncRNA was screened, including 11 up-regulated lncRNA and 22 down- regulated lncRNA, which was shown in heat map (Figure 1A). And the OIP5-AS1 expression level which was down-regulated in CRC cells with 2 Gy dose of radiation compared with cells without x-ray irradiation (Figure 1A). Subsequently, results of qRT-PCR verified that the expression levels of OIP5-AS1 were remarkably lower in radio-resistant

cells (LoVo-RA and SW480-RA) than in parental cells (LoVo and SW480) ($P < 0.01$, Figure1B). In clonogenic survival assay, radioresistance of irradiated cells (LoVo-RA and SW480-RA) was obviously stronger than that of parental cells (LoVo and SW480) (Figure 1C), indicating that radio-resistant cells were successfully established. In addition, both mRNA and protein expressions of *DYRK1A* were notably lower in radio-resistant cells than that in untreated parental cells (Figure 1D-1E). Kaplan-Meier method was used analyze the information obtained from TCGA database and the overall survival (OS) curve was produced. Low expression of OIP5-AS1 and *DYRK1A* was correlated with a poor survival (Figure 1F).

OIP5-AS1 targeted miR-369-3p and suppressed the expression of miR-369-3p

According to the prediction of miRDB database, OIP5-AS1 could target miR-369-3p (Figure 2A). In dual luciferase reporter assay, we constructed the reporters containing OIP5-AS1-WT or OIP5-AS1-Mut and co-transfected them with miR-369-3p mimics into HEK293T cells. Results showed that compared with the negative control group, relative luciferase activity of OIP5-AS1-WT was significantly weakened in cells transfected with miR-369-3p mimics ($P < 0.05$, Figure 2B), validating the targeting relationship between OIP5-AS1 and miR-369-3p. To investigate whether OIP5-AS1 could impact miR-369-3p expression in radio-resistant CRC cells, OIP5-AS1 was transfected into LoVo-RA and SW480-RA cells. Results of qRT-PCR demonstrated that OIP5-AS1 was successfully up-regulated in radio-resistant cells, while relative expression levels of miR-369-3p remarkably decreased in OIP5-AS1 overexpression group compared with the mock group ($P < 0.05$, Figure 2C-2D). These results revealed that OIP5-AS1 could interact with miR-369-3p and overexpression of OIP5-AS1 suppressed miR-369-3p expression in radio-resistant CRC cells.

MiR-369-3p directly targeted *DYRK1A* and inhibited the expression of *DYRK1A*

According to the prediction of miRDB database, miR-369-3p could directly target *DYRK1A* (Figure 3A). Similarly, we constructed luciferase reporters containing *DYRK1A*-UTR-WT or *DYRK1A*-UTR-Mut and co-transfected them with miR-369-3p mimics into HEK293T cells. Results

of dual luciferase reporter assay demonstrated that the luciferase ability of *DYRK1A* wild-type was considerably weaker in miR-369-3p mimics group than in the negative control group ($P < 0.05$, Figure 3B). Furthermore, *DYRK1A*'s expression in two radio-resistant cell lines (LoVo-RA and SW480-RA) remarkably elevated after cell transfection with OIP5-AS1, whereas notably reduced after transfection with miR-369-3p mimics ($P < 0.05$, Figure 3C). However, co-transfection of OIP5-AS1 and miR-369-3p (MIX group) showed no significant influence on *DYRK1A* expression ($P > 0.05$, Figure 3C). The above results indicated that miR-369-3p inhibited *DYRK1A* expression in radio-resistant CRC cells by targeting regulation, and meanwhile, that miR-369-3p overexpression attenuated the promotive effects of OIP5-AS1 overexpression on *DYRK1A* expression.

DYRK1A regulated the radioresistance of CRC cells

DYRK1A siRNA was transfected into parental cell line LoVo so that the protein expression of *DYRK1A* was down-regulated, while *DYRK1A* was transfected into radio-resistant cell line LoVo-RA to up-regulate *DYRK1A* expression ($P < 0.01$, $P < 0.001$, Figure 4A-4B).

According to clonogenic survival assay, *DYRK1A* knockdown in LoVo cells could increase the surviving fraction, whereas overexpression of *DYRK1A* in LoVo-RA cells reduced cellular survival (Figure 4C), suggesting that *DYRK1A* enhanced the radiosensitivity of CRC cells. Each group was then irradiated with a dose of 6 Gy x-ray radiation for 24 h. Results of MTT assay showed that cell viability in LoVo+si-*DYRK1A* group was dramatically stronger than that in LoVo group, while cell viability in LoVo-RA+*DYRK1A* notably declined compared with LoVo-RA group and the same trends were shown 48 h after irradiation ($P < 0.001$, Figure 4D).

Without irradiation, down-expression of DYRK1A observably inhibited apoptosis of LoVo cells while over-expression of DYRK1A increased the apoptosis rate in LoVo-RA cells, indicating that DYRK1A could control CRC apoptosis in a radiation independent way. After 6 Gy x-ray irradiation, percentage of apoptotic cells in each group apparently increased compared with that before irradiation. In addition, similar to the above results of without irradiation, DYRK1A observably

induced apoptosis in LoVo and LoVo-RA cells after irradiation ($P < 0.01$, $P < 0.001$, Figure 5A). Further we explored the possible mechanism of DYRK1A on apoptosis in LoVo and LoVo-RA cells with or without irradiation. The level of DYRK1A, p-P53 (S15), total P53, cleaved caspase-9 and cleaved caspase-9 were measured by western blot (Figure 5B). The DYRK1A expression level was decreased in si-DYRK1A transfection group and increased in DYRK1A transfection group with or without x-ray irradiation (Figure 5C).Down-expression of DYRK1A observably reduced the level of p-P53 (S15), but not total P53 protein with or without x-ray irradiation, indicating that DYRK1A induced apoptosis may be involved in P53 pathway in a radiation independent way (Figure 5D). After irradiation, the level of cleaved caspase-9 and caspase-3 both were up-regulated in LoVo cells compared with no irradiation group. Down-expression of DYRK1A also resulted in the inhibition of caspase-9 and caspase-3 in LoVo cells with or without irradiation, and up-expression of DYRK1A increased cleaved caspase-9 and caspase-3 level in LoVo-RA cells with or without irradiation (Figure 5E, D). Taken together, *DYRK1A* impeded cell viability, promoted radio-induced cell apoptosis, and enhanced the radiosensitivity of CRC cells.

OIP5-AS1 influenced the radiosensitivity of CRC cells through *DYRK1A*

To further explore the impact of OIP5-AS1 on the radiosensitivity of CRC cells, we successfully established OIP5-AS1 down-regulation cells in LoVo cell lines (LoVo+si-OIP5-AS1 group) and OIP5-AS1 overexpression cells in LoVo-RA cell lines (LoVo-RA+OIP5-AS1 group) ($P < 0.05$, $P < 0.01$, Figure 6A). Results of qRT-PCR and western blot showed that *DYRK1A* expression was considerably lower in LoVo+si-OIP5-AS1 group than in LoVo group and remarkably higher in LoVo-RA+OIP5-AS1 group than in LoVo-RA group ($P < 0.01$, Figure 6B).

After irradiation, LoVo cells were easier to survive after knocking down OIP5-AS1 as the surviving fraction in LoVo+si-OIP5-AS1 group was higher compared with LoVo group, while the viability of LoVo-RA cells was suppressed as the surviving fraction in LoVo-RA+OIP5-AS1 group was lower

compared with LoVo-RA group (Figure 6C), indicating the reinforcing effect of OIP5-AS1 on the radiosensitivity of CRC cells. Silencing of OIP5-AS1 also improved cell viability of LoVo cells irradiated with a dose of 6 Gy x-ray for 24 h ($P < 0.001$, Figure 6D). In contrast, overexpression of OIP5-AS1 reduced viability of radio-resistant LoVo cells (LoVo-RA) ($P < 0.001$, Figure 6D). Meanwhile, cells irradiated for 48 h showed similar trends in viability as those received 24 h irradiation ($P < 0.001$, Figure 6D).

Cell apoptosis rate in each group dramatically declined after irradiation. Under the same conditions (with or without irradiation), percentage of apoptotic cells in LoVo+si-OIP5-AS1 group was lower than that in LoVo group, while apoptotic cells in LoVo-RA+OIP5-AS1 group was much more than that in LoVo-RA group ($P < 0.05$, $P < 0.01$, $P < 0.001$, Figure 6E). These results indicated that lncRNA OIP5-AS1 could impede cell viability, promote radio-induced cell apoptosis, and enhance radiosensitivity of CRC cells through regulating *DYRK1A* expression.

Discussion

The negative influence lncRNA OIP5-AS1 imposed on radioresistance of CRC cells was identified in this study, and the relevant mechanism regulated by miR-369-3p and *DYRK1A* was also described. To be specific, overexpression of OIP5-AS1 degraded the expression of miR-369-3p, leading to the upregulation of miR-369-3p's target gene *DYRK1A*, which could restrain viability, induce apoptosis and enhance radiosensitivity of CRC cells. Therefore, OIP5-AS1 reduced radioresistance of CRC cells through regulating miR-369-3p and *DYRK1A*.In radio-resistant CRC cells, we found the distinctively lower expression levels of OIP5-AS1 and *DYRK1A* than that in normal CRC cells, implying that OIP5-AS1 and *DYRK1A* might impair radioresistance. OIP5-AS1 is a lncRNA required for proper mitotic cell division (Kim et al., 2017), and it was reported to be up-regulated in some types of cancers, such as hepatocellular carcinoma (Li et al., 2017) and glioma tissues (Hu et al., 2017). Here we demonstrated the low expression of OIP5-AS1 in radio-resistant

CRC cells for the first time, indicating that it was associated with radioresistance of CRC.

Radiotherapy is a standard preoperative treatment approach for local advanced cancer to reduce local recurrence (Wang et al., 2014). Numerous lncRNAs plays a key role in regulating tumor signal pathway, thereby affecting tumorigenesis and progression from many different aspects, including proliferation, invasion, migration or radiosensitivity. Wang *et al.* identified the down-regulated lncRNA p21 in CRC cells and proved that enforcing its expression could enhance radiosensitivity (Wang et al., 2014). The down-regulation of HOTAIR has been shown to improve the radiosensitivity of CRC cells (Yang et al., 2016). OIP5-AS1 might have similar effect as lncRNA p21 and HOTAIR. Zhang *et al.* reported that lncRNA CRNDE function as an oncogene by modulating p21, contributing to the radioresistant phenotype formation of Human Lung Adenocarcinoma. OIP5-AS1 might have similar effect as lncRNA p21 and HOTAIR. Besides, the aberrantly low expression of *DYRK2* was found in CRC tissues and cells (Wang et al., 2017) and predicted poor prognosis (Yan et al., 2016), which was consistent with our findings about *DYRK1A*.

Then we inspected the target relationships among lncRNA OIP5-AS1, miR-369-3p and *DYRK1A*, and confirmed that OIP5-AS1 targeted miR-369-3p and suppressed its expression, while miR-369-3p down-regulated *DYRK1A*'s expression. The interactions between lncRNAs and miRNAs in cancer cells tremendously contributed to the tumor progression or regression (Wang et al., 2015). OIP5-AS1 could inhibit cell proliferation in multiple myeloma (Yang et al., 2017) and cervical carcinoma (Kim et al., 2016) by mediating the expressions of miR-410 and miR-424, respectively. MiRNAs could further regulate their target genes to influence tumor development. According to previous studies, *DYRK1A* was down-regulated by miR-1246 to regulate cell cycle and reduce apoptosis (Liao et al., 2012; Yang et al., 2015). Similarly, our study revealed that in CRC, OIP5-AS1 could bind to miR-369-3p, which further targeted *DYRK1A*, providing a novel insight

into the regulatory effect of OIP5-AS1 in CRC.

Since OIP5-AS1 could increase *DYRK1A*'s expression indirectly, their biological functions in CRC were studied in details. Both of them impeded CRC deterioration by restraining cell viability, inducing apoptosis, and reducing radioresistance. Studies on OIP5-AS1 and *DYRK1A*'s functions in CRC were limited. According to the researches conducted by Li *et al.* and Gong *et al*, silencing of OIP5 inhibited cell growth of clear cell renal cell carcinoma (Gong et al., 2013) and hepatocellular carcinoma (Li et al., 2017), and reduced metastasis. On the contrary, in cervical carcinoma, it played a suppressive role as a sponge for proliferation related miRNA or RNA-binding protein (Kim et al., 2016; Yang et al., 2017). Therefore, OIP5-AS1's effect varied with different types of cancers. In CRC, it acted as a sponge or a competing endogenous to mediate miR-369-3p, thus up-regulating the tumor suppressive gene *DYRK1A*. The biological functions of *DYRK1A* were also dependent on cancer types. Depletion of *DYRK1A* in gastrointestinal stromal tumors enhanced chemosensitivity (Boichuk et al., 2013), while overexpression of *DYRK1A* in acute myeloid leukemia achieved similar effects (Liu et al., 2014). In CRC, *DYRK1A* served as a tumor suppressor because of its promotive effect on radiosensitivity of CRC cells.

We also explored the effect of *DYRK1A* on apoptosis in colorectal cancer. Down-expression of *DYRK1A* observably reduced phosphorilation of P53. The similar results were found by Joongkyu *et al*. that DYRK1A-induced p53 phosphorylation at Ser-15 in embryonic neuronal cells [PMID: 20696760]. *DYRK1A* also promote apoptosis in mitochondrial pathway by up regulated cleaved caspase-9 and caspase-3 level. Laguna *et al*. reported that *DYRK1A* regulates caspase-9-mediated apoptosis during retina development [PMID: 19081073]. .Although our study uncovered the functions of OIP5-AS1 and *DYRK1A* in CRC and attempted to explain the mechanism, the results were only obtained from *in vitro* experiments. Lack of *in vivo* experiments was one of the

limitations of this study. Meanwhile, clinical analysis is worth to be supplemented to determine the diagnostic and prognostic values of OIP5-AS1, miR-369-3p and *DYRK1A*.

In summary, OIP5-AS1 and *DYRK1A* were both down-regulated in radio-resistant CRC cells. Overexpression of OIP5-AS1 could down-regulate miR-369-3p's expression in CRC, thus enhancing the expression of *DYRK1A*. OIP5-AS1 and *DYRK1A* suppressed proliferation and promoted radio-induced apoptosis of CRC cells. Therefore, overexpression of OIP5-AS1 could significantly reduce radioresistance of CRC cells by regulating *DYRK1A* through miR-369-3p.

Funding: This study was supported by National Science Youth Foundation Grant No. 81502118 for Yanmei Zou; National Science Foundation Grant No. 81772827 for Yihua Wang; Natural Science Foundation of Hubei Province No. 2016CKB711 for Hua Xiong.

Conflict of interest: The authors declare no potential conflicts of interest.

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Figure legends

Figure 1. Radiotherapy decreased the expression of OIP5-AS1 and *DYRK1A* in CRC cells. (A) Differentially expressed lncRNAs in two cell lines (Lovo and SW480) with or without exposure to 2 Gy dose of radiation. OIP5-AS1 expression was down-regulated in irradiated cells. (B) Relative expression levels of OIP5-AS1 were lower in radio-resistant cell lines (LoVo-RA and SW480-RA) than in parental cell lines (LoVo and SW480). (C) Results of clonogenic survival assay showed that radioresistance of irradiated cells (LoVo-RA and SW480-RA) was stronger than parental cells (LoVo and SW480). (D-E) MRNA and protein expression levels of DTRK1A were lower in radio-resistant cell lines (LoVo-RA and SW480-RA) than in parental cell lines (LoVo and SW480). (F) Survival analysis showed that lower OIP5-AS1 or *DYRK1A* expressing CRC had a poorer

prognosis. *, $P < 0.05$. **, $P < 0.01$.

Figure 2. OIP5-AS1 regulated miR-369-3p expression in radio-resistant CRC cells. (A) The predicted binding site in OIP5-AS1 wild-type and miR-369-3p from miRDB database. (B) The luciferase activity of OIP5-AS1-WT was weakened after transfected with miR-369-3p mimics, while the luciferase activity of OIP5-AS1-Mut in cells transfected with miR-369-3p mimics showed no significant difference from that in the negative control group. (C) Results of qRT-PCR showed that OIP5-AS1 was successfully up-regulated in radio-resistant cells LoVo-RA and SW480-RA. (D) Results of qRT-PCR showed that expression levels of miR-369-3p were notably lower in OIP5-AS1 overexpression group than in the mock group. *, $P < 0.05$.

Figure 3. MiR-369-3p inhibited *DYRK1A* expression by targeting regulation. (A) The predicted binding site in miR-369-3p and *DYRK1A* 3'UTR wild-type from miRDB database. (B) The luciferase activity of *DYRK1A*-UTR-WT was weakened in cells transfected with miR-369-3p mimics, while the luciferase activity of *DYRK1A*-UTR-Mut in cells transfected with miR-369-3p mimics showed no significant difference from that in the negative control group. (C) *DYRK1A* expression dramatically increases in OIP5-AS1 overexpression cells, but remarkably decreased in miR-369-3p overexpression cells. *DYRK1A* expression showed no significant difference in cells transfected with both OIP5-AS1 and miR-369-3p mimics from that in the mock group. *, $P < 0.05$.

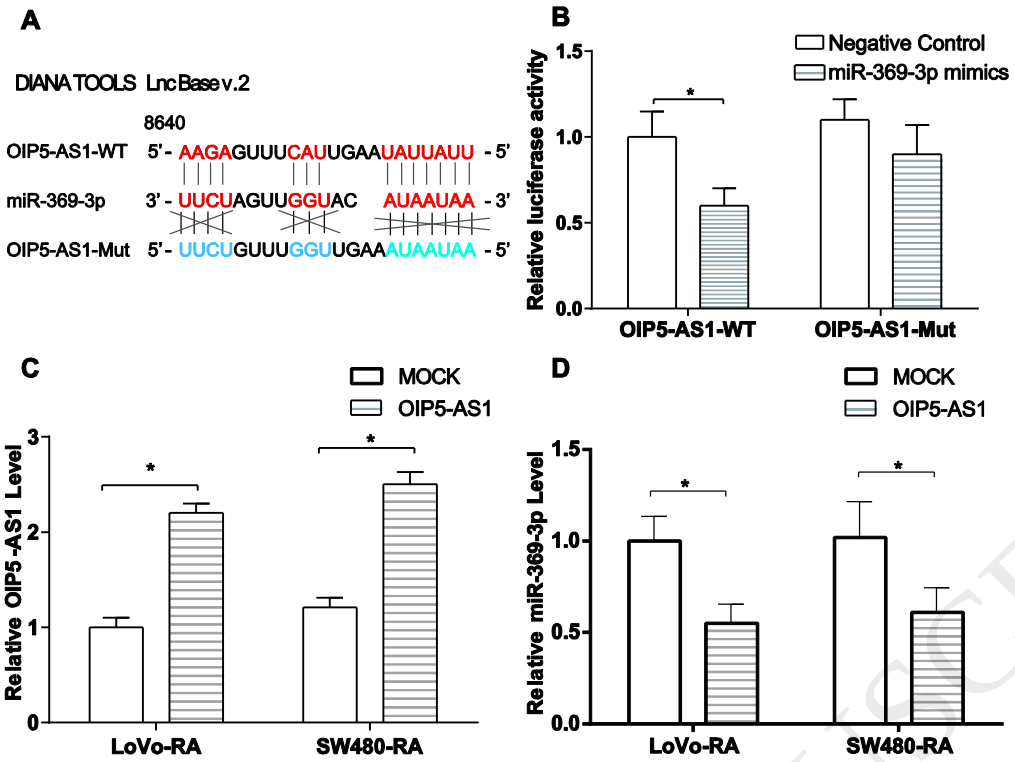
Figure 4. *DYRK1A* influenced cell viability and radioresistance of CRC cells. (A-B) Results of western blot verified that *DYRK1A* expression was down-regulated in LoVo+si-*DYRK1A* group and up-regulated in LoVo-RA+*DYRK1A* group. (C) Results of conogenic survival assay showed that the surviving fraction of cells in LoVo+si-*DYRK1A* group was notably higher than that in LoVo group but lower in LoVo–RA+*DYRK1A* group than in LoVo-RA group. (D) Results of MTT assay showed that cell viability dramatically increased in LoVo+si-*DYRK1A* group compared with that in LoVo

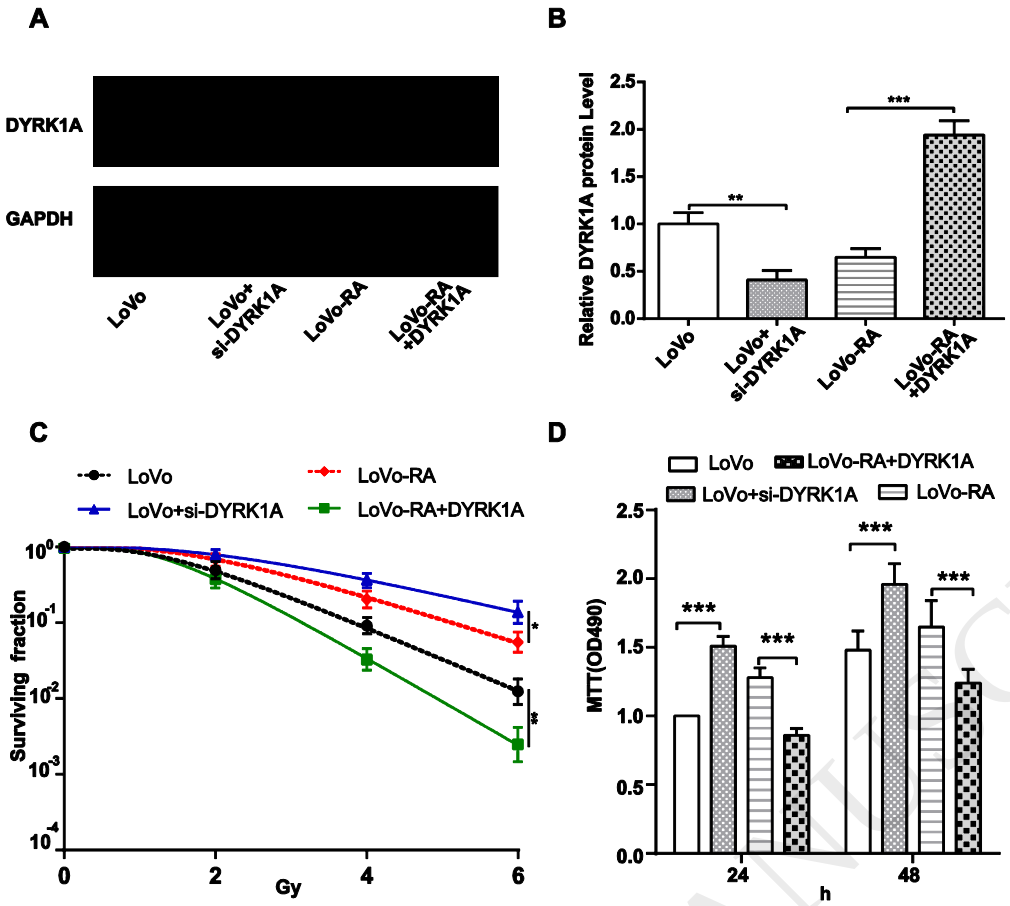
group but declined in LoVo-RA+*DYRK1A* compared with LoVo-RA group. *, $P < 0.05$. **, $P < 0.01$. ***, $P < 0.001$.

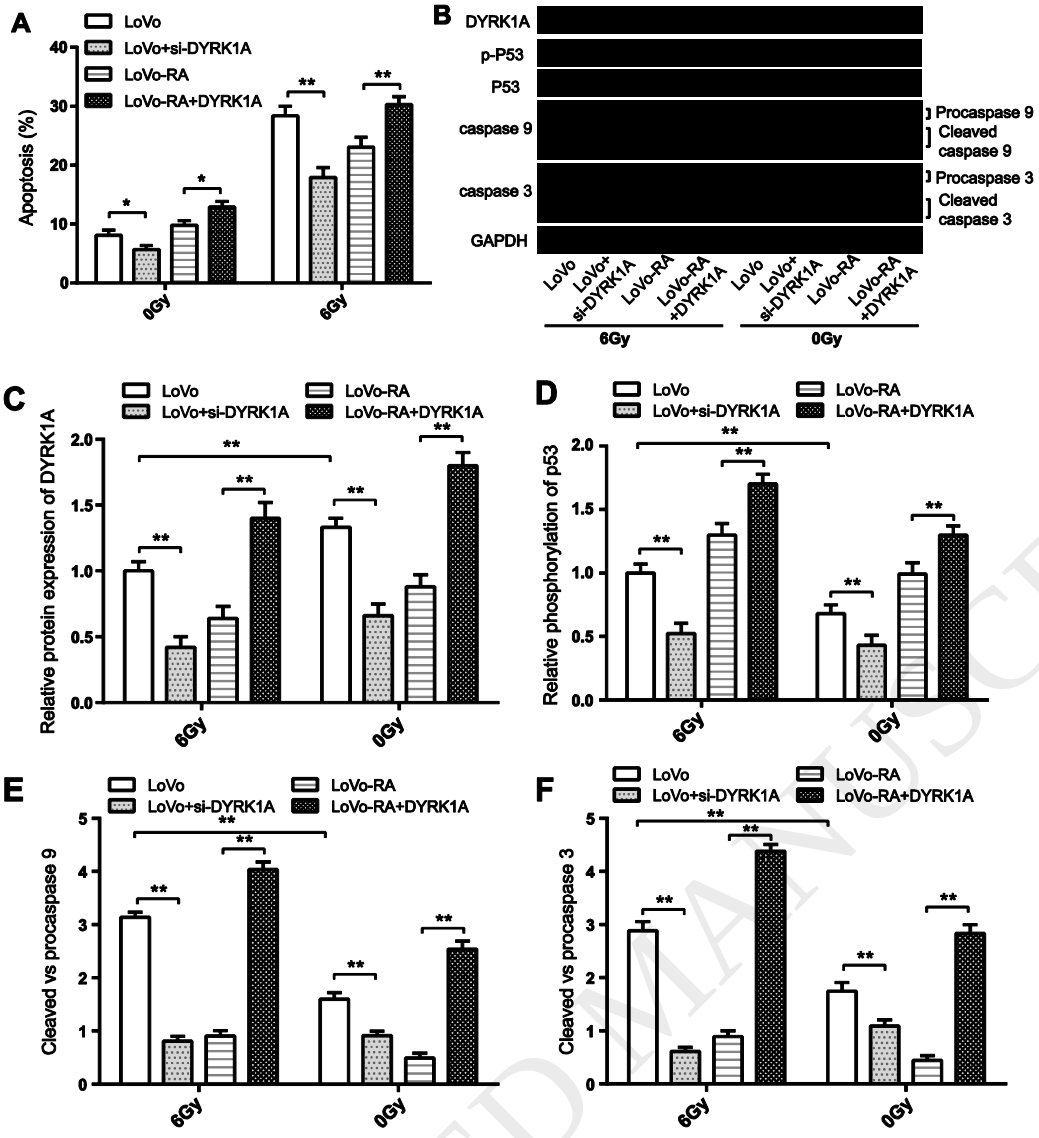
Figure 5. *DYRK1A* influenced cell apoptosis and radioresistance of CRC cells (A) Under the same conditions (with or without irradiation), percentage of apoptotic cells in LoVo+si-*DYRK1A* group was dramatically lower than that in LoVo group while higher in LoVo-RA+*DYRK1A* group than that in LoVo-RA group. Percentage of apoptotic cells in groups with exposure to 6 Gy x-ray irradiation apparently increased compared with groups without irradiation. (B) Western blot analysis was carried out for the indicated proteins using LoVo and LoVo-RA cells exposured to 0 Gy or 6 Gy x-ray irradiation. (C) Quantitation of DYRK1A expression of different groups. (D) Quantitation of phosphorylation of P53, the data was normalized by LoVo cells with 6Gy. (E) Quantitation of cleaved caspase 3 of different groups. (F) Quantitation of cleaved caspase 9 of different groups. **, $P < 0.01$. ***, $P < 0.001$.

Figure 6. OIP5-AS1 influenced cell viability and radioresistance of CRC cells. (A) Results of qRT-PCR verified that OIP5-AS1 expression was down-regulated in LoVo+si-OIP5-AS1 group and up-regulated in LoVo-RA+OIP5-AS1 group. (B) Results of qRT-PCR and western blot showed that *DYRK1A* expression was down-regulated in LoVo+si-OIP5-AS1 group compared with LoVo group and up-regulated in LoVo-RA+OIP5-AS1 group compared with LoVo-RA group. (C) Results of conogenic survival assay showed that the surviving fraction of cells in LoVo+si-OIP5-AS1 group was remarkably higher than that in LoVo group, while the surviving fraction in LoVo-RA+OIP5-AS1 group was much lower than that in LoVo-RA group. (D) Results of MTT assay showed that cell viability in LoVo+si-OIP5-AS1 was significantly stronger than that in LoVo group, while cell viability in LoVo-RA+OIP5-AS1 was weakened compared with LoVo-RA group. (E) Under the same conditions (with or without irradiation), cell apoptosis rate in

LoVo+si-OIP5-AS1 group was obviously lower than that in LoVo group, while cell apoptosis rate in LoVo-RA+OIP5-AS1 group was much higher than that in LoVo-RA group. Cell apoptosis rates in groups with exposure to 6 Gy x-ray irradiation apparently higher than those in groups without irradiation. *, $P < 0.05$. **, $P < 0.01$. ***, $P < 0.001$.







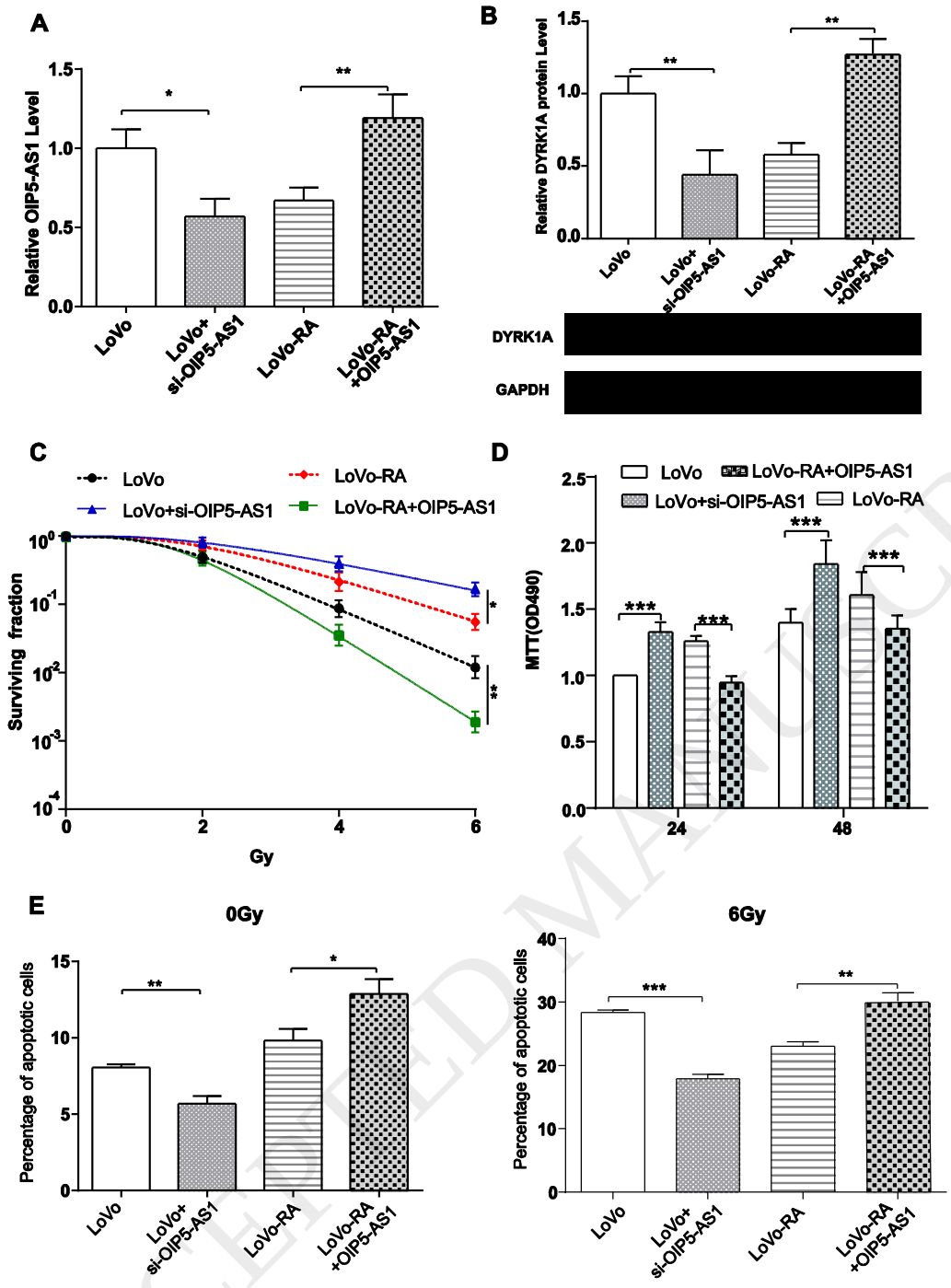


Table 1 Primers for qRT-PCR.

	Primers
GAPDH-F	5'- TGTGGGCATCAATGGATTTGG-3’
GAPDH-R	5’- ACACCATGTATTCCGGGTCAAT-3’
OIP5-AS1-F	5’- TGCGAAGATGGCGGAGTAAG-3’

OIP5-AS1-R	5’- TAGTTCCTCTCCTCTGGCCG-3’
DYRK1A-F	5’- GCAATTTCCTGCTCCTCTTG-3’
DYRK1A-R	5’- TTACCCAAGGCTTGTTGTCC-3’
miR-369-3p-F	5'- TGGGAATAATACATGGTTGATC -3'
miR-369-3p-R	5'- CAGTGCGTGTCGTGGAGT -3'
U6-F	5’-CTTCGGCAGCACATATAC-3’
U6-R	5’-GAACGCTTCACGAATTTGC-3’

F, forward; R, reverse.