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Promotion of Radioresistance of RACGAP1 in Digestive System Malignancy Through Impact on Cell Senescence

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Abstract

Background: *RACGAP1 (Rac GTPase Activating Protein 1) belongs to RHOGAP, has been proved to be an oncogene in several tumors. Aims: In this study, we attempted to validate the promotion of radioresistance of RACGAP1 in digestive system malignancy and tried to uncover potential mechanism. Methods and results: Firstly, bioinformatics analyses for public datasets were taken. Then a series of in vitro experiments including CCK8 assay, Transwell migration and invasion experiments, and plate cloning formation experiment were conducted on digestive system malignancy. Finally, proteomics through iTRAQ (Isobaric tags for relative and absolute quantitation, iTRAQ) technology on colorectal cancer cell line LS411N with RACGAP1 knocked down was conducted and following bioinformatic analyses were carried out. In this study, we found that RACGAP1 is high expressed in digestive system malignancy and has negative effect on survival. RACGAP1 promotes proliferation, migration and invasion of pancreatic cancer in vitro. RACGAP1 promotes radioresistance in pancreatic cancer, esophageal squamous carcinoma and colorectal cancer in vitro. Proteomics of colorectal cancer cell line LS411N with RACGAP1 knocked down shows differentially expressed proteins got enriched in pathway of cell senescence.*

Conclusion: *RACGAP1 is a promising oncogene target for digestive system malignancy. RACGAP1 might promote radioresistance through its impact on cell senescence.*

Keywords: Rac GTPase Activating Protein 1, Radioresistance, Cell Senescence, Digestive System Malignancy, Protein Mass Spectrometry

Introduction

Malignant tumors of the digestive system refer to cancers occurring in various regions of the digestive tract, including the esophagus, stomach, liver, pancreas, small intestine, and colon. In recent years, the incidence of gastrointestinal malignancies has steadily increased, making them a critical focus in the field of oncology. Among all malignant tumor systems, digestive system cancers rank first in both incidence (24%) and mortality (35%)[1]. This upward trend is closely associated with factors such as sedentary lifestyles, poor dietary habits, and an aging population [2].

It is crucial to identify the shared characteristics and treatment challenges of gastrointestinal malignant tumors. These tumors are typically aggressive, with a pronounced tendency for metastasis. Furthermore, the majority of patients are diagnosed at advanced stages, which severely restricts treatment options and results in a poor prognosis. Standard therapies—such as surgery, chemotherapy, and radiation—have demonstrated varying degrees of success. Nevertheless, the heterogeneity and resistance mechanisms inherent in gastrointestinal malignant tumors often curtail the effectiveness of these approaches. This underscores the pressing need to discover novel therapeutic targets to address

current treatment limitations effectively. Small GTPases of the RHO family (RHO GTPases) are intracellular signaling molecules that belong to the RAS superfamily.

Under normal physiological conditions, the activity of these proteins is tightly regulated by three distinct types of molecules: guanine nucleotide exchange factors (GEFs), GTPase-activating proteins (GAPs), and guanine nucleotide dissociation inhibitors (GDIs). GEFs activate RHO GTPases by facilitating the exchange of GDP for GTP, while GAPs deactivate these proteins by enhancing their intrinsic GTPase activity, converting them back to their GDP-bound inactive state. GDIs, on the other hand, help maintain RHO GTPases in a stable, inactive state within the cytoplasm and enable their ready translocation to the membrane, where nucleotide exchange occurs. Once activated, RHO GTPases transmit signals to a wide array of effectors, thereby regulating cytoskeletal dynamics and key cellular processes such as cell cycle progression, cytokinesis, migration, and polarity [3].

Unlike RAS GTPases, which are mutated in approximately 27% of human cancers, mutations in RHO proteins are relatively rare. Instead, RHO proteins more frequently contribute to cancer development through dysregulation of their expression or activation[4]. Historically, it was widely believed that RHO GTPases and RHOGEFs functioned as oncogenic drivers, with these proteins expected to show elevated expression levels in malignant tumors, while RHOGAPs, as tumor suppressor proteins, were thought to exhibit reduced expression. However, recent studies have challenged this view, revealing that certain RHOGAPs, despite their presumed tumor-suppressive role, are highly expressed in malignant tumors and exhibit carcinogenic properties.

RACGAP1 (Rac GTPase Activating Protein 1) belongs to RHOGAP, and current research has found that its expression level is increased in breast cancer[3], hepatocellular carcinoma[5], gastric cancer[6], esophageal squamous cell carcinoma[7], melanoma[8], high-grade meningioma[9], prostate cancer[10], colorectal cancer[11] and other malignant tumors, which promotes the occurrence and development of tumors and is associated with poor prognosis. In pancreatic cancer, RACGAP1 was also found to be associated with poor prognosis and its expression correlated with the migration and metastasis ability of pancreatic cancer cells [12, 13].

In their study, Ping-Hsiu Wu et al. observed an elevated expression of the RACGAP1 protein in several cell lines, including human breast cancer (MDA-MB-231), human head and neck tumor (FaDu), human pancreatic cancer (PANC-1), and mouse breast cancer (4T1), following X-ray irradiation[14]. These findings suggest a potential association between RACGAP1 and the radioresistance of malignant tumors.

However, the specific role of RACGAP1 in tumor radioresistance has not yet been elucidated. Therefore, this study aimed to investigate the contribution of RACGAP1 to radioresistance in pancreatic cancer, esophageal squamous carcinoma, and colorectal cancer. Additionally, the underlying mechanisms were preliminarily explored using protein mass spectrometry after silencing RACGAP1 in the colorectal cancer cell line LS411N.

Methods

Bioinformatics Analyses on Public Datasets

For differential analysis, The Cancer Genome Atlas Program (TCGA) data sets of esophageal cancer, gastric cancer, colorectal cancer, liver cancer, pancreatic cancer, and biliary tract cancer were downloaded from UCSC xena database (https://xena.ucsc.edu/public), and expression levels of genes were calculated with log2 (FPKM+1). RACGAP1 expression data were extracted from the above datasets, and boxplots of differences between groups were plotted using the R package "ggpubr", and significance was calculated between groups using Wilcox.

For survival analysis, the median expression level of RACGAP1 in each tumor sample was used to classify the high and low expression groups, and the R package survival was used to draw the survival curve. To analyze the effect of irradiation on expression of RACGAP1, dataset GSE104965 was downloaded from the GEO database (https://www.ncbi.nlm.nih.gov/geo/). In this dataset, pancreatic cancer cells were seeded into culture plate overnight with 2% FBS in culture medium and then were irradiated with 10Gy using an Oncor linear accelerator. Irradiated pancreatic cancer cells were collected at 24h after X-ray radiation for RNA extraction and hybridization on Affymetrix microarray. The author sought to obtain whole transcript profiles in pancreatic cancer cells treated with X-ray radiation. There were three samples in control and experiment groups, respectively. R package "limma" was used for differential analysis of the dataset, and the threshold of differential genes was $|logFC|>0.5$ and $P\ value<0.05$

RACGAP1 siRNA Synthesis and Transfection

siRNA target sequences were identified on the human RACGAP1 sequence. Using online tool oligowalk, siRNA sequence was designed as follows: (sense) 5'-GCGAAGUGCUCUGGAUGUU-3'. These siRNA and negative control (NC) sequences were produced from Ribobio (Guangzhou, China). When cells were at approximately 50% confluency, the siNC and siRNA were transfected into the cells, using Lipofectamine 3000 (Invitrogen), according to the manufacturer's instructions.

RNA Preparation and Quantitative PCR Amplification

Total RNA was extracted from PANC-1 cell line, using the EZ-press RNA Purification Kit (EZBioscience) according to the manufacturer's instructions, and quantified. An One-Step gDNA Removal and cDNA Synthesis SuperMix (TransGen Biotech) was used for reverse transcription of RNA into cDNA. The primers (Ribobio (Guangzhou, China)) RACGAP1 Forward: 5'-TCTCAACAGAGGCCAACCATCC-3', Reverse: 5'-ACTGCAGAGCCAATGGAACGAG-3' and GAPDH Forward:5'-GAACGGGAAGCTCACTGG-3', Reverse:5'-TCTTCCACCACTTCGTCCG-3', were designed to amplify cDNA with Green One-Step qRT-PCR SuperMix (TransGen Biotech). PCR conditions were 95°C for 2 min, 95°C for 15 sec, and 60°C for 30 sec for 40 cycles. The relative amount of RACGAP1 mRNA was normalized to that of GAPDH. The $2^{-\Delta\Delta C}$ method was used to calculate this.

Western Blot Analysis

Cells were incubated in lysis buffer (Beyotime), with 1% PMSF for 30 minutes on ice to obtain total lysates. 30 minutes later, the lysed cells were centrifuged at a temperature of 1 000 × g for

10 minutes at 4 ° C, and the supernatants were extracted with a BCA Protein Assay kit (Thermo Fisher Scientific, Inc.) for a period of 10 minutes by addition of 5 times SDS. To the resulting 12% SDS-PAGE gel for electrophoresis separation, the total protein content (50 µg) was added and transferred to 0.45 µm PVDF film (Amersham Hybond, GE Health). Then, the membrane was blocked for 2 hours with 1% of bovine serum (Amresco). Then, RACGAP1 (1: 1000, 66056-1-lg; Proteintech), GAPDH (1: 50000, 60004-1-Ig; Proteintech) was used for incubation. The membrane was washed for 1 hour with TBS-T (0.1% Tween 20) for a period of 10 minutes, and then washed with Goat Anti-Mouse IgG (H + L) (1: 2000, SA00001-1; Protein Technology). The membranes were then exposed to a chemiluminescent substrate (Thermo Scientific), followed by exposure to a Syngene imaging device (Syngene Imaging Systems).

Cell Culture, Exposure to Radiation and Plate Cloning Formation Experiment

The pancreatic cancer cell line PANC-1, esophageal squamous carcinoma cell line KYSE150 and colorectal cancer cell line HCT-8 and LS411N were acquired from the National Collection for Authenticated Cell Cultures (Shanghai, China). The cells were cultured in a moist, 5% CO2 incubator at 37 ℃, and they were grown in DMEM using 10% FBS and 100 U/mL penicillin/streptomycin. Cells were exposed to single doses of 4 Gy and 8 Gy X-rays. The growth curve of the cells was observed using the cck-8 experiment. Additionally, clonogenic cell numbers were calculated following exposures to single fraction with 0, 2, 4, 6, 8, and 10Gy X-rays. The cell survival curve was then fitted using the single-hit multi-target model SF=1 - (1 - e-kD)N.

Cell Proliferation, Migration and Invasion Assay

Cell proliferation was measured by CCK-8 (TransDetect® Cell Counting Kit, FC101-01, Beijing, China). The cells were seeded in a 96 well plate at a density of 1000 cells per well, followed by incubation at 37ºC and 5% carbon dioxide. After 1, 2, 3, 4, 5, 6, 7 days, the culture solution was discarded, and a 10% CCK-8 serum free medium was added. After 2 hours of incubation, the cell proliferation was estimated with a microplate reading device (Bio-Tek).

In the migration experiment, 3×104 cells were seeded in a Transwell insert (8 mm pore size; Corning Inc.) in a serum-free medium, and 20% FBS was added as a chemoattractant in the lower chamber. In the invasion experiment, Matrix (BD Bioscience) was applied to 8×104 cells in the upper chamber, and 20% of FBS in the lower chamber. After incubation for 48 hours at 37°C and 5% CO2, Transwell chamber was removed, and the medium was removed from the well and washed with calcium-free PBS. Then, the cells were fixed for 30 minutes with methanol and stained for 20 minutes with 0.1% crystalline violet. Using a cotton swab, the upper non-migrating cells were lightly rubbed and counted in the microscope.

Proteomics of Colorectal Cancer Cell Line LS411N

RACGAP1 expression of colorectal cancer cell line LS411N was knocked down by siRNA interference technique and the control group was transfected with negative control sequences. Isobaric tags for relative and absolute quantitation (iTRAQ) were used to quantitatively analyze samples. The experiment was repeated for 3 times in both groups. Differential proteins were defined as those with |Fold change|>1.2 and P-value<0.05. Cluster analysis, GO, KOG, Pathway functional enrichment analysis, PPI analysis and other bioinformatics analysis were performed.The iTRAQ (Isobaric tags for relative and absolute quantitation, iTRAQ) technology has proved to be successful in numerous experimental contexts. This quantitative method can be used for measure eight samples at one experiment and has the characteristics of high precision. So far, it has been more and more widely used in the field of quantitative proteomics [15]. The iTRAQ (Isobaric tags for relative and absolute quantitation, iTRAQ) technology was provided and conducted in BGI Genomics (Shenzhen, China).

Statistical Analysis

Statistical analysis was carried out with GraphPad Prism 9 software. The data were represented by the mean ± standard deviation (SD), and the analysis was done using one way ANOVA or Student T test. The difference in P value < 0.05 was regarded as statistically significant.

Results

Bioinformatics Analyses on Public Datasets

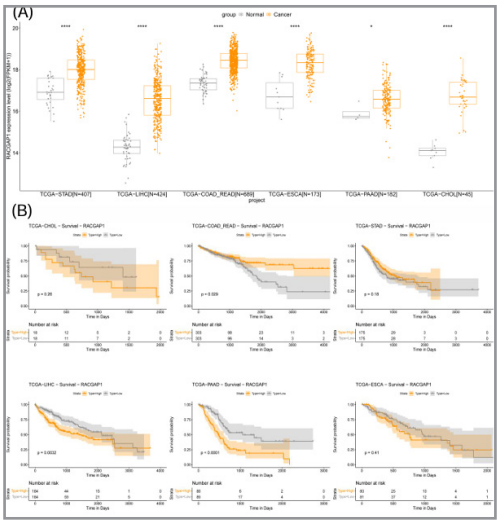


Figure 1: As shown in Figure 1(A), in TCGA dataset, RACGAP1 was significantly up-regulated in esophageal (ESCA), gastric (STAD), colorectal (READ_COAD)

liver (LIHC), pancreatic (PAAD), and biliary tract (CHOL) cancers in tumor samples compared to normal samples. Survival information was available in 36 of 36 cancer samples in CHOL, in 606 of 638 cancer samples in READ_COAD, in 350 out of 375 cancer samples in STAD, in 368 of 374 cancer samples in LIHC, in 177 out of 178 cancer samples in PAAD, in 161 out of 162 cancer samples in ESCA. Figure 1(B) shows that groups with high RACGAP1 expression have significantly worse survival than that with low RACGAP1 expression in liver (LIHC) and pancreatic (PAAD) cancer.

In GSE104965, a total of 22 differentially expressed genes between irradiated and nonirradiated pancreatic cancer cells were identified by setting the screening threshold (|logFC|>0.5 and P-value<0.05), including 9 up-regulated and 13 down-regulated genes. To understand the difference of gene distribution on the whole, using volcano figure to show the distribution is shown in figure 2

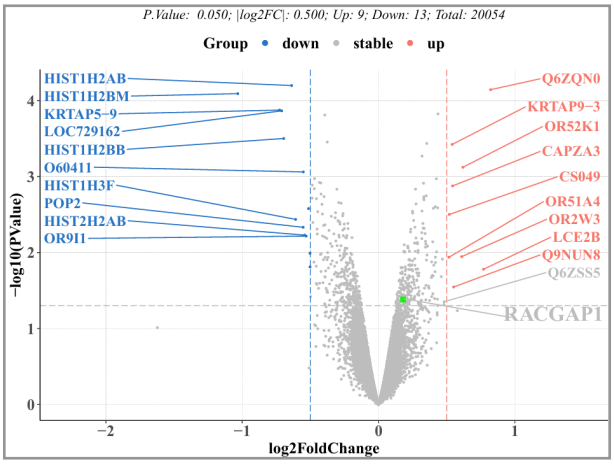


Figure 2: Among them, the expression of RACGAP1 was up-regulated in irradiated pancreatic cancer cells, although with no significance.

RACGAP1 Promotes Proliferation, Migration and Invasion of Pancreatic Cancer

RACGAP1 was effectively knocked down in PANC-1 Cells by transfection of siRNA at mRNA as well as protein level (Figure 3A). CCK8 experiment showed that the proliferation of PANC-1 cells was slowed down after interference of RACGAP1 by siRNA, which infested that RACGAP1 promote proliferation of pancreatic cancer cell line PANC-1 (Figure 3B).Transwell migration experiments showed reduced PANC-1 cells passing through the chamber of the siRACGAP1 group (Figure 3C). Transwell invasion experiments showed reduced PANC-1 cells passing through the chamber after interference of RACGAP1 by siRNA (Figure 3D)

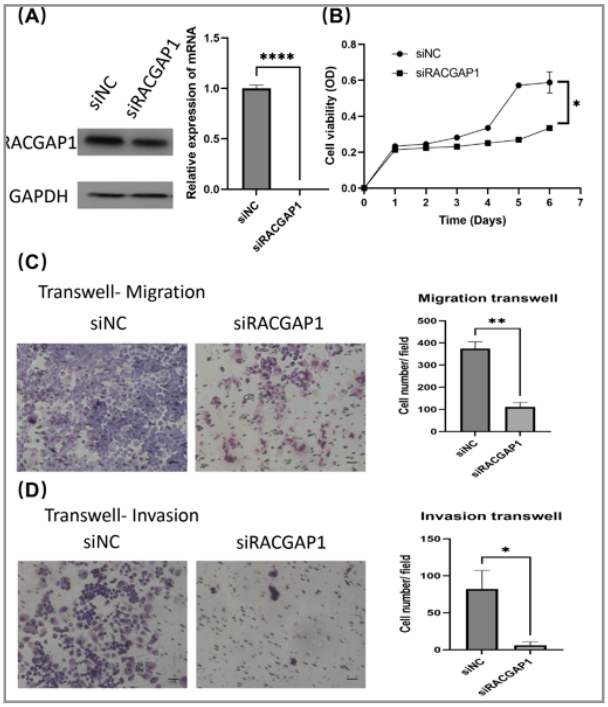
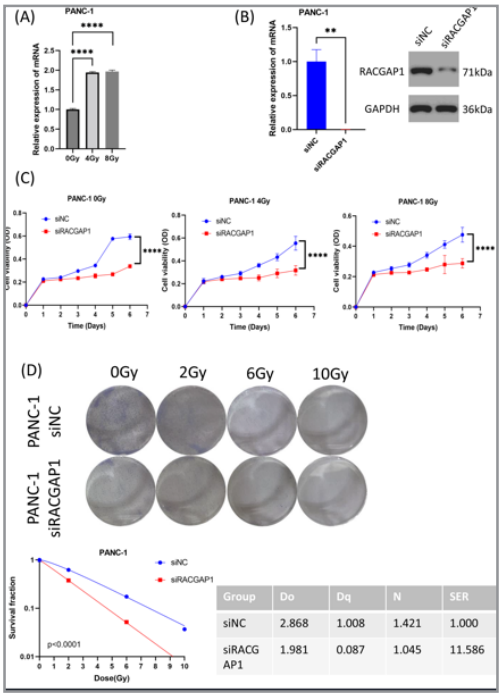


Figure 3: It could be inferred that RACGAP1 played an important part in migration and invasion ability of PANC-1.

RACGAP1 Promotes Radioresistance in Pancreatic Cancer, Esophageal Squamous Carcinoma and Colorectal Cancer

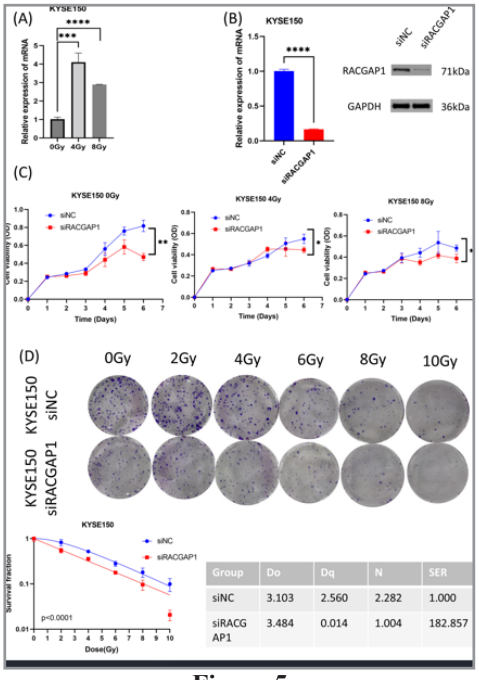
The mRNA expression level of RACGAP1 in pancreatic cancer cell line PANC-1 was significantly increased 24h after 4Gy and 8Gy X-ray irradiation (Figure 4A). After successfully knocking down mRNA and protein expression of RACGAP1 using siRNA interference technology (Figure 4B), CCK8 experiment showed that the proliferation ability of cell lines was significantly inhibited after 4Gy and 8Gy X-ray irradiation (Figure 4C). The plate cloning formation experiment showed that the plate cloning ability of the cell line after X-ray irradiation was significantly inhibited. Its fitted cell survival fraction curve was significantly reduced, and the sensitizing enhancement ratio (SER) was greater than 1, indicating that the radiosensitivity of the pancreatic cancer cell line PANC-1 was enhanced after RACGAP1 knocked down (Figure 4D).



Group	Do	Dq	N	SER
siNC	2.868	1.008	1.421	1.000
siRACGAP1	1.981	0.087	1.045	11.586

Figure 4

The mRNA expression level of RACGAP1 in esophageal squamous carcinoma cell line KYSE150 was significantly increased 24h after 4Gy and 8Gy X-ray irradiation (Figure 5A). After successfully knocking down mRNA and protein expression of RACGAP1 using siRNA interference technology (Figure 5B), cck-8 experiment showed that the proliferation ability of cell lines was significantly inhibited after 4Gy and 8Gy X-ray irradiation (Figure 5C). The plate cloning formation experiment showed that the plate cloning ability of the cell line after X-ray irradiation was significantly inhibited. Its fitted cell survival fraction curve was significantly reduced, and the sensitizing enhancement ratio (SER) was greater than 1, indicating that the radiosensitivity of KYSE150 was enhanced after RACGAP1 knocked down (Figure 5D).



Group	Do	Dq	N	SER
siNC	3.103	2.560	2.282	1.000
siRACGAP1	3.484	0.014	1.004	182.857

Figure 5

The mRNA expression level of RACGAP1 in colorectal cancer cell line HCT-8 was significantly increased 24h after 4Gy and 8Gy X-ray irradiation (Figure 6A). After successfully knocking down mRNA and protein expression of RACGAP1 using siRNA interference technology (Figure 6B), cck-8 experiment showed that the proliferation ability of cell lines was significantly inhibited after 4Gy and 8Gy X-ray irradiation (Figure 6C). The plate cloning formation experiment showed that the plate cloning ability of the cell line after X-ray irradiation was significantly inhibited. Its fitted cell survival fraction curve was significantly reduced, and the sensitizing enhancement ratio (SER) was greater than 1, indicating that the radiosensitivity of HCT-8 was enhanced after RACGAP1 knocked down (Figure 6D).

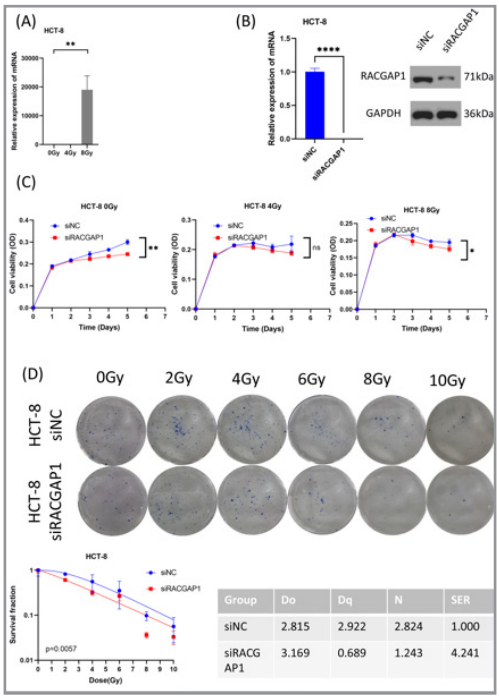


Figure 6

Proteomics of Colorectal Cancer Cell Line LS411N

A total of 305 differentially expressed proteins (DEPs) were identified. Compared to the LS411N-siNC group, 89 proteins were significantly upregulated, while 216 proteins were downregulated in the LS411N-siRACGAP1 group. The expression of RACGAP1 protein was significantly reduced in the siRACGAP1 group, consistent with the experimental results. Protein enrichment analysis is a technique used to examine protein expression data. It involves applying statistical methods, based on existing knowledge—specifically proteome annotation information—to identify the primary functions of various proteins. This approach enables researchers to swiftly comprehend the functional roles associated with the research sample and its corresponding protein set. The key bioinformatics analysis results are summarized as follows.

Subcellular Localization Prediction of DEPs

Protein targeting, also known as protein sorting, is a biological process by which proteins are directed to their proper destinations within the cell or outside of it. These destinations may include the interior of an organelle, various intracellular membranes, the plasma membrane, or the extracellular environment when secreted. This precise transportation process relies on information encoded within the protein itself. Accurate protein sorting is essential for maintaining cellular function, as errors in this process can result in the development of diseases. We perform protein subcellular localization prediction using bioinformatic tools (WoLF PSORT[16] for eukaryotes).

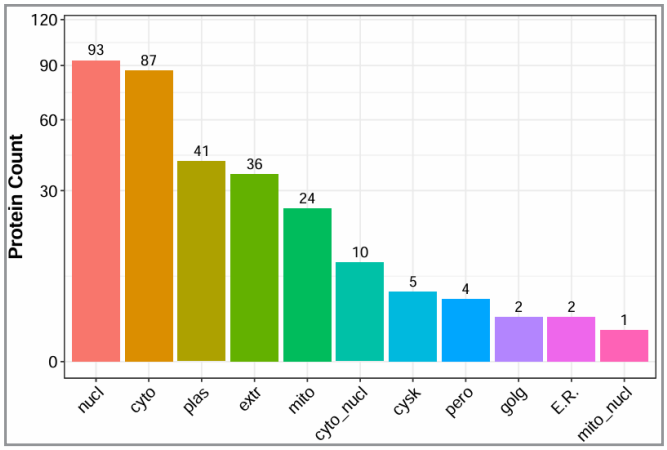


Figure 7

Figure 7 illustrates the predicted subcellular localization of the differentially expressed proteins (DEPs). The majority of DEPs are situated in the nucleus and cytoplasm. These findings highlight variations in expression regulation between the two groups.

GO Enrichment Analysis of DEPs

The GO enrichment analysis highlights the Gene Ontology (GO) terms associated with differentially expressed proteins among all identified proteins. This analysis reflects the key biological functions relevant to the project, providing valuable insights into its underlying mechanisms.

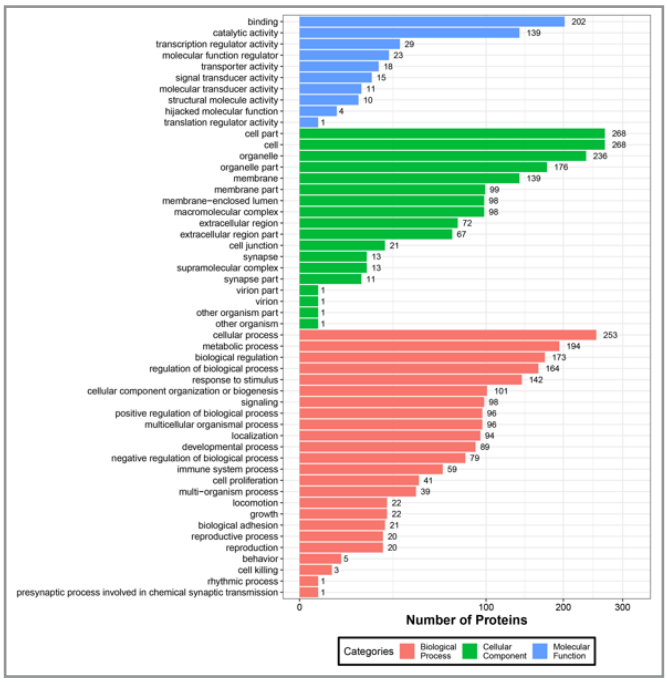


Figure 8

Figures 8 and 9 present the Gene Ontology analysis of DEPs. Figure 8 illustrates the GO functional classification map of the total DEPs.

The analysis reveals that the total DEPs are most significantly enriched in binding (molecular function), cell part and cell (cellular component), and cellular process (biological process).

Figure 9 illustrates the GO functional classification map for up-regulated and down-regulated DEPs. Notably, both up- and down-regulated DEPs exhibit a similar GO enrichment pattern. They are enriched in categories such as cellular processes, metabolic processes, cell, cell parts, binding, and catalytic activity, among others. 4.3 KOG Annotation of DEPs For the differentially expressed proteins, their corresponding KOG terms were extracted, and several bar plots were generated as a result. This approach allows us to easily identify and categorize their functional classifications.

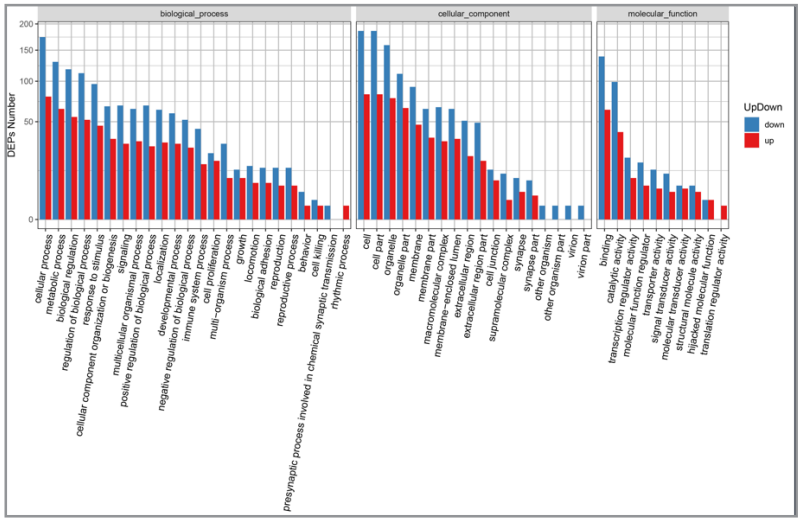


Figure 9

Figure 10 illustrates the KOG annotation of DEPs. The DEPs are primarily enriched in signal transduction mechanisms (cellular processes and signaling), transcription (information storage and processing), and inorganic ion transport and metabolism (metabolism).

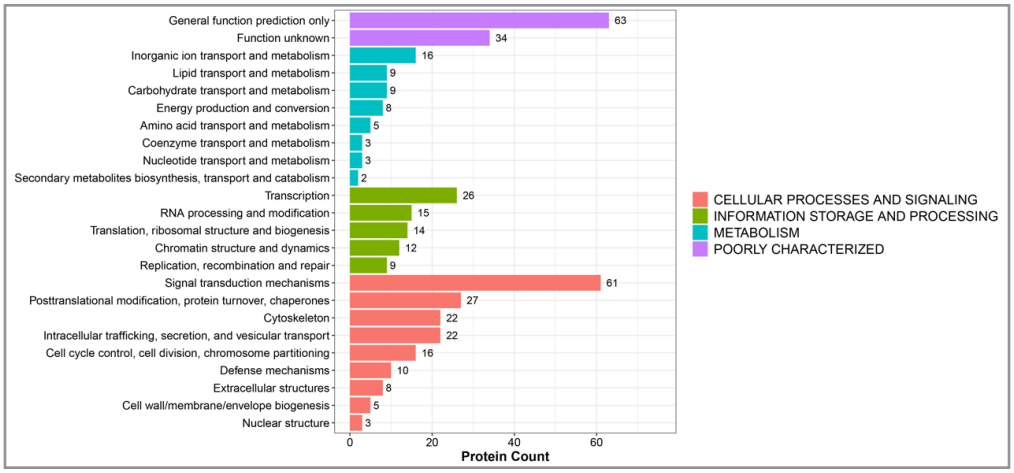


Figure 10

Pathway Enrichment Analysis of DEPs

Proteins often interact with each other to fulfill specific biological functions. To explore these interactions, we conduct pathway enrichment analysis of differentially expressed proteins using the KEGG database. For each pairwise comparison, we compile a comprehensive HTML report detailing these differentially expressed proteins. Furthermore, we create a scatter plot highlighting the top 20 KEGG enrichment results, presented as Figure 11.

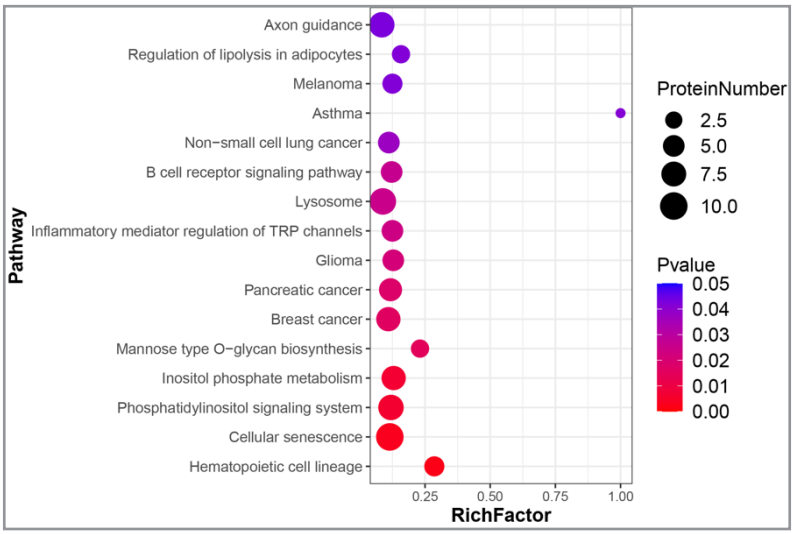


Figure 11

The 16 most enriched pathways identified were hematopoietic cell lineage, cellular senescence, the phosphatidylinositol signaling system, inositol phosphate metabolism, mannose-type O-glycan biosynthesis, breast cancer, pancreatic cancer, glioma, inflammatory mediator regulation of TRP channels, lysosome, B cell receptor signaling pathway, non-small cell lung cancer, asthma, melanoma, regulation of lipolysis in adipocytes, and axon guidance.

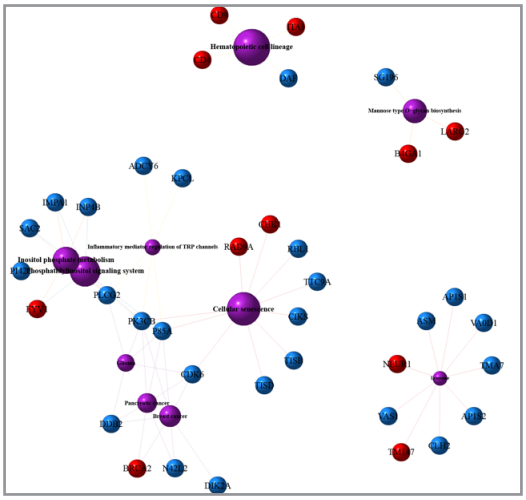


Figure 12

Figure 12 illustrates the network analysis of pathway terms. Notable overlaps were observed among several enrichment pathways, including breast cancer, pancreatic cancer, glioma, cellular senescence, inflammatory mediator regulation of TRP channels, inositol phosphate metabolism, and the phosphatidylinositol signaling system.

We then shifted our focus to the cellular senescence pathway. The differentially expressed proteins (DEPs) identified within this pathway included RBL1 (down-regulated), TISB (down-regulated), TISD (down-regulated), CHK1 (up-regulated), TTC9A (down-regulated), CIKS (down-regulated), RAD9A (up-regulated), CDK6 (down-regulated), P85A (down-regulated), and PK3CB (down-regulated).

As shown in Figure 13, we further present these DEPs within the pathway map of cell senescence.

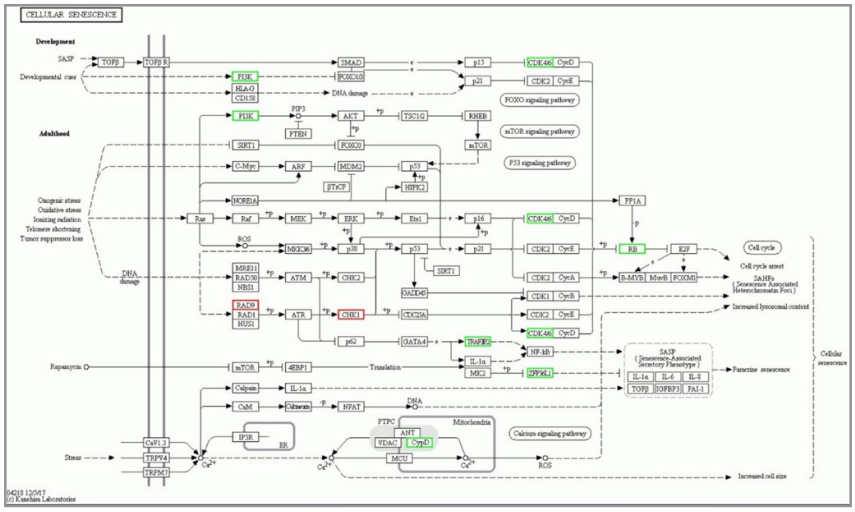


Figure 13

Protein-Protein Interaction of DEPs

STRING is a comprehensive database that catalogues both known and predicted protein-protein interactions (PPIs) [17]. These interactions encompass direct (physical) connections as well as indirect (functional) associations. The data is derived from computational predictions, knowledge transfer across different organisms, and aggregated interactions sourced from other primary databases.

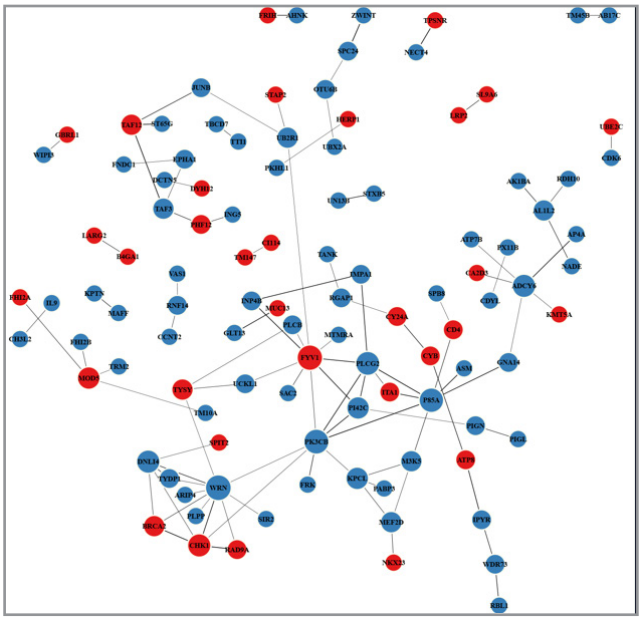


Figure 14

Figure 14 illustrates the protein-protein interactions of differentially expressed proteins (DEPs) based on the STRING database. The analysis reveals that the DEPs predicted to directly interact with RACGAP1 are TANK, which is downregulated, and CY24A, which is upregulated.

Discussion

Ping-Hsiu Wu and colleagues observed that the expression level of RACGAP1 protein was significantly elevated in human breast cancer MDA-MB-231 cells, human head and neck tumor FaDu cells, human pancreatic cancer PANC-1 cells, and mouse mammary cancer 4T1 cells following X-ray irradiation [14]. Similarly, in our study, we also identified a pronounced increase in RACGAP1 expression in human pancreatic cancer PANC-1 cells, human esophageal squamous cell carcinoma KYSE150 cells, and human colon adenocarcinoma HCT-8 and LS411N

cells after irradiation. These findings suggest that RACGAP1 may play a role in contributing to radioresistance in malignant tumors.

Firstly, we demonstrated through in vitro experiments that RACGAP1 plays a significant role in promoting the proliferation, migration, and invasion of pancreatic cancer. These results were subsequently corroborated by proteomics analyses conducted on the colorectal cancer cell line LS411N, where differentially expressed proteins (DEPs) were found to be enriched in several prominent cancer pathways, including those associated with breast cancer, pancreatic cancer, glioma, non-small cell lung cancer, and melanoma.

Furthermore, knockdown of RACGAP1 using siRNA in PANC-1, KYSE150, HCT-8, LS411N, and SW480 cells significantly reduced plate colony formation and inhibited cell proliferation following X-ray irradiation. These in vitro experiments conclusively demonstrate that RACGAP1 enhances the radioresistance of malignant tumors in the digestive system. To investigate the downstream molecular mechanisms, we employed siRNA to suppress RACGAP1 expression in LS411N colorectal cancer cells, subsequently performing proteomic analysis and bioinformatics evaluations. Our findings revealed that the differentially expressed proteins were significantly enriched in the cellular senescence pathway. This observation led us to hypothesize that RACGAP1 enhances radioresistance in colorectal cancer by mediating cellular senescence.

Cell senescence is a state of permanent cell cycle arrest that causes significant changes in cell morphology and physiology. These changes include an increase in cell size and irregularity in shape, upregulation of caveolin-1, and the removal of DPP4 from the cell membrane. Additionally, there is an upsurge in lysosomal activity, characterized by an increase in lysosomes and lysosomal proteins, as well as a buildup of mitochondria. Alterations in the nucleus, such as the depletion of LaminB1, are also observed. The third edition of the cancer hallmarks, introduced in 2022, offers a framework for evaluating these markers to determine the extent of cell senescence in tumors [18].

Cellular senescence is one of the outcomes induced by ionizing radiation (IR). Exposure to IR can result in DNA damage in both tumor and normal cells. Non-homologous end joining (NHEJ) and homologous recombination (HR) are the two primary pathways for repairing double-strand breaks (DSBs), which are particularly potent triggers for inducing cellular senescence (CS) [19]. This study explores the fundamental principles of radiotherapy, with DNA being the primary cellular target of IR. DNA damage can occur either through direct ionization or indirectly via hydroxyl radicals (OH•), resulting in base modifications, disruption of the sugar-phosphate backbone, and the formation of single-strand breaks (SSBs), double-strand breaks (DSBs), or DNA cross-links.

The effects of radiation with different energy densities are different: high-LET radiation mainly acts directly; while low-LET radiation has an indirect-to-direct ratio of about 3:1. After DNA damage, radiation-induced cellular responses include cell cycle arrest, cell apoptosis, mitotic dysfunction, autophagy, and cell senescence[20]. The accuracy of DNA damage repair by related downstream signaling pathways determines cell fate, including senescence and apoptosis etc[21]. Generally, DNA double-strand breaks (DSBs) are an especially potent stimulus for inducing cellular senescence (CS) [22]. IR-induced CS (IRIS) is also a form of stress-induced premature senescence (SIPS) [23], which can occur in a few cell types, including cancer cells, fibroblasts, epithelial cells, endothelial cells (ECs), immune cells, and stem cells. Senescent cells (SCNs) are characterized by apoptosis resistance, metabolic activity, proinflammatory and profibrotic molecule secretion, and alterations in the surrounding microenvironment, despite the absence of cell division capacity and permanent arrest of proliferation[24]. Ionizing radiation activates p53 and subsequent p21 expression, and permanent G1 blockade leads to cell senescence [20].

It has been reported that ionizing radiation (IR) can elicit a number of different cellular responses, including immunogenic apoptosis, necroptosis, mitotic catastrophe and senescence. In order to establish a correlation between a cellular outcome and a specific dose of irradiation, Sandy Adjemian et al. conducted a comprehensive investigation in a panel of cell lines, examining the cellular responses at varying doses of X-rays. It is noteworthy that all cell lines tested exhibited a comparable response to IR, displaying characteristics of mitotic catastrophe, senescence, lipid peroxidation, and caspase activity [25]. The IR dose plays a pivotal role in the induction of senescence or apoptosis upon cell exposure. A low dose (0.5-10 Gy) of IR induces senescence, while a high dose (>10 Gy) induces apoptosis [26]. This phenomenon is related to the level of DNA damage and the functionality of the DDR network. Therefore, the fraction regimen also appears to affect IRIS and may respond differently in different cells [19].

However, nowadays it is still controversial on the relationship between CS and radiosensitivity. Although many radiosensitizers function based on CS appeared such as Poly (ADP-ribose) polymerase (PARP) inhibitor [27] and inhibition of the mammalian target of rapamycin (MTOR) pathway etc. [28], limited benefits suggest that more complicated mechanisms should be considered and explored because CS may facilitate radioresistance in tumour cells. The phenomenon of senescent cells (SCNs) regrowing following exposure to IR has recently attracted increasing attention. This suggests that CS plays 'opposing roles' in RT and other genotoxic therapies[29, 30]. Some DNA damage foci induced by IR may persist for an extended period. However, the repair of DSBs in SCNs may ultimately result in recovery and regrowth following combination IR/PARPi treatment [31, 32].

Furthermore, cells regrown after IR-induced CS (IRIS) may exhibit more aggressive biological behaviours, such as enhanced proliferative ability and increased invasion and migration capacities, than those existing before IR. Additionally, SCNs demonstrated the capacity to secrete a multitude of factors that facilitate growth and invasion in vitro and in vivo[33]. Collectively, although our understanding of CS in IR is still initial, similar to RT in the treatment of cancer, IRIS functions as a'double-edged sword'and crucially influences the comprehensive results of RT. Campbell D. Lawson et al. found that depletion of RacGAP1 in basal-like breast cancers cells inhibited growth through the combined effects of cytokinesis failure, CDKN1A/p21-mediated RB1 inhibition, and the onset of senescence [34].

We also discovered that DEPs got enriched in pathway of cellular senescence after knocking down RACGAP1 with siRNA, which is consistent with their findings. Therefore, we proposed the idea that RACGAP1 promotes radioresistance in colorectal cancer through inhibition of cellular senescence. In the proteomic and bioinformatics analysis of cellular senescence, we found that PI3K (downregulated)-CDK6 (downregulated)-RBL1 (downregulated) were enriched in the cellular senescence pathway as shown in pathway enrichment analysis of DEPs part (Figure 11). Yang Gu et al. validated that proliferation through the PI3K/AKT/CDK2 and PI3K/AKT/GSK3β/Cyclin D1 signaling pathways [35].

In the study by Lei Zhang et al., transcriptional analysis showed that CDK6 gene knockout regulates the downregulation of PI3K110α/110β expression; PI3K110α/110β deficiency leads to the downregulation of CDK6 expression; therefore, cancer cells exhibit a CDK6-PI3K axis cooperative action [36]. This evidence implied a potential link on signaling pathway between proteins PI3K, CDK6 and RBL1. Based on these, we predict that RACGAP1 inhibits cellular senescence and promotes radiation resistance in colorectal cancer through the PI3K-CDK6-RBL1 pathway. Due to the complexity of the role of cell senescence in radiosensitivity, more in-depth and comprehensive researches are warranted to validate its detailed mechanism.

Conclusion

RACGAP1 is a promising oncogene target for digestive system malignancy. RACGAP1 might promote radioresistance through its impact on cell senescence.

Declarations

Data Availability Statement

The data that supports the findings of this study are openly available in Science Data Bank (https://www.scidb.cn/en) at https://doi.org/10.57760/sciencedb.12319, reference number 12319.

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Conflict of Interest Disclosure

The authors declare that they have no competing interests.

Ethics Approval Statement

Not applicable.

Patient Consent Statement

Not applicable.

Permission to Reproduce Material from Other Sources

Not applicable.

Clinical Trial Registration

Not applicable.

Authors' Contributions

All authors had full access to the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Conceptualization, Chunyan Qiu; Methodology, Chunyan Qiu and Jingni Zeng; Formal Analysis, Chunyan Qiu and Jingni Zeng; Resources, Chunyan Qiu; Writing - Original Draft, Chunyan Qiu and Jingni Zeng; Writing - Review & Editing, Chunyan Qiu; Visualization, Chunyan Qiu; Funding Acquisition, Chunyan Qiu.

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