

中文摘要

Cofilin-1 在人结直肠癌细胞辐射应答中的作用研究

结直肠癌（Colorectal Cancer，CRC），作为我国最常见的癌症之一，其发病率及死亡率一直以来都位居恶性肿瘤前列。CRC 产生除遗传因素外，还受生活方式（如抽烟、喝酒、久坐等）、饮食习惯（高脂饮食、缺乏膳食纤维等）多种因素的影响。近年来社会发展导致我国国民生活作息的变动，也让 CRC 由 50 岁以上高发的老年患者逐渐向青年、中年群体转移。早期 CRC 可直接手术切除，但大多患者确诊 CRC 时已经处于中晚期，此时极易发生周围组织浸润和远处转移，因而常结合放疗用于术前术后照射提高疗效、清扫病灶周围淋巴结和晚期的姑息治疗。但由于 CRC 具有较高的辐射抗性，单纯放疗作用下降，严重影响了放疗患者的预后。

丝切蛋白 Cofilin-1 作为 ADF/Cofilin 家族的一员，能特异性结合肌动蛋白丝并切割生成 G-actin 从而调节肌动蛋白动力学，由此通过重塑细胞骨架参与调节细胞。细胞骨架作为真核生物细胞中最广泛存在的细胞成分之一，参与多种生命活动，如细胞迁移、细胞增殖、周期调控、凋亡坏死、损伤修复等。已有实验报道人类部分恶性肿瘤细胞中 Cofilin 异常表达，且 Cofilin-1 参与了具有辐射抗性的癌细胞的 DNA 修复从而影响肿瘤患者放疗预后。但结直肠癌细胞辐射后的生物效应与 Cofilin-1 在其中发挥的作用仍不完全清楚。因此本研究以人结直肠癌细胞为主要研究对象，以 Cofilin-1 为目标，探究 Cofilin-1 对人结直肠癌细胞的辐射应答反应中的调节作用及可能的机制。

目的：

以 Cofilin-1 蛋白为研究切入点，通过 cytochalasin D（以下简称 cytoD）的 Cofilin-1 抑制调节作用，探究 Cofilin-1 在人结直肠癌细胞中辐射生物效应中的作用，从而为优化结直肠癌放疗方案和临床诊断治疗提供理论依据。

方法:

1. 分别用 0.1 (0.0134 Gy/min) 和 2、4、6、8 Gy (1.02 Gy/min) 剂量的 X 射线辐照 HCT116、HT29 细胞, 在照射后的 24 h 收集细胞, WB 检测辐射对其 Cofilin-1 及其磷酸化蛋白的表达影响。

2. 通过 CCK8 法检测上述剂量 X 射线辐射下 24 h 后结直肠癌细胞的增殖能力。

3. 使用 phalloiding 鬼笔环肽染色法检测结直肠癌细胞的骨架变化。

4. 采用 γ H2AX 免疫荧光法检测结直肠癌细胞的 DNA 损伤水平。

5. 使用 JC-1 探针法检测结直肠癌细胞线粒体膜电位。

6. 通过 DCFH-DA 探针法检测辐照前后结直肠癌细胞 ROS 生成水平。

7. 通过流式细胞术检测结直肠癌细胞周期变化。

8. 采用流式细胞术检测结直肠癌细胞自噬水平。

9. 通过流式细胞术检测结直肠癌细胞凋亡水平。

结果:

1. 电离辐射对结直肠癌细胞增殖率和 Cofilin-1 表达的影响

与对照组 (0 Gy) 相比, 照射后 24 h, HCT116 细胞的增殖能力随剂量的增高而表现出降低的趋势, 其中剂量 6 Gy 为时趋势最明显。与对照组 HCT116 细胞相比, 照射后 Cofilin-1 表达总体呈降低趋势, 磷酸化 Cofilin-1 表达在大剂量 (6、8 Gy) 时呈升高趋势, 且 6 Gy 时磷酸化 Cofilin-1 表达升高约 0.5 倍, 差异最大。

与对照组 (0 Gy) 相比, 照射后 24 h, HT29 细胞的增殖能力在 2、4 Gy 时与 HCT116 细胞相似呈降低的趋势, 但在 6、8 Gy 时 HT29 细胞增殖能力反而随剂量升高而增强 (仍低于各自分组的对照组)。与对照组 HT29 细胞相比, Cofilin-1 表达无显著差异; 磷酸化 Cofilin-1 表达总体呈升高趋势, 且在 6 Gy 时趋势最明显 (表达提升约 1 倍)。

2. cytoD 联合电离辐射对结直肠癌细胞骨架稳态的影响

Phalloidin 染色后, 对照组 HCT116 细胞边界完整清晰, 但胞质内并无明显的骨架纤维丝, 这可能与肿瘤细胞的旺盛增殖有关; 6 Gy 照射后 24 h 可以诱

导 HCT116 细胞应激修复反应,从而出现稳定细胞状态的应力纤维;cytoD($1\ \mu\text{M}$)作用 48 h 后可以明显破坏细胞骨架的原有结构,染色显示肌动蛋白散乱分布;联合处理后 HCT116 细胞并未出现明显的应力纤维,结果说明 cytoD 作用抑制了 Cofilin-1 解聚肌动蛋白的功能,使 HCT116 细胞在接受照射后骨架无法及时修复,这可能与 Cofilin-1 表达减少细胞无法清除骨架断点和提供足量 G-actin 有关。HT29 相对于 HCT116 整体而言细胞直径偏小,染色后无法观测到明显的应力纤维,但结果仍显示出 cytoD 对细胞骨架完整性的破坏效果。

3. cytoD 联合电离辐射对结直肠癌细胞 DNA 损伤的影响

γH2AX 免疫荧光染色显示,6 Gy 照射后 2 h 和 cytoD ($1\ \mu\text{M}$) 作用 24 h 后部分 HCT116 细胞核内出现焦点损伤,而二者联合处理后细胞核中出现焦点损伤的 HCT116 细胞数量显著提升 ($P<0.05$); HT29 细胞染色结果与 HCT116 细胞趋势一致,其中 cytoD 作用后效果与照射相比更明显 ($P<0.05$)。这些结果说明电离辐射和 cytoD 作用均能导致结直肠癌细胞 DNA 损伤,二者联合作用时结直肠癌细胞 DNA 损伤加剧。

4. cytoD 联合电离辐射对结直肠癌细胞线粒体膜电位去极化效果的影响

JC-1 免疫荧光染色显示,6 Gy 照射后 2 h 和 cytoD ($1\ \mu\text{M}$) 作用 24 h 后,部分 HCT116 细胞荧光从高电位的红色荧光逐渐表现为低电位的绿色荧光 ($P<0.05$),而二者联合处理提高了 HCT116 细胞绿-红荧光的比值,即 HCT116 细胞表现出更强的线粒体膜电位去极化效果 ($P<0.05$); HT29 细胞与 HCT116 细胞趋势相似,其中加药组比照射组线粒体膜电位去极化效果影响更低 ($P<0.05$)。这些结果可能是由于电离辐射和 cytoD 损伤细胞线粒体膜导致的线粒体膜电位去极化。

5. cytoD 联合电离辐射对结直肠癌细胞 ROS 积累的影响

DCFH-DA 探针检测 ROS 显示,6 Gy 照射后 2 h 和 cytoD ($1\ \mu\text{M}$) 作用 24 h 后,与 HCT116 细胞对照组相比,部分细胞出现 ROS 的绿色荧光信号 ($P<0.05$),而联合处理组中细胞绿色荧光信号强度增强更明显 ($P<0.05$); HT29 细胞也呈现出与 HCT116 细胞相同的趋势。其缘由除电离辐射直接导致细胞 ROS 生成外,

还可能因 cytoD 作用导致线粒体膜破坏、内源性 ROS 释放和细胞整体 ROS 清除障碍有关。

6. cytoD 联合电离辐射对结直肠癌细胞周期的影响

使用流式细胞术检测各组 HCT116 细胞周期，6 Gy 照射后 24 h 和 cytoD (1 μ M) 作用 48 h 后，与对照组细胞相比，处理组 HCT116 细胞出现显著的 G2 期阻滞 ($P<0.05$)，其中药物作用比电离辐射更明显 ($P<0.05$)；HT29 细胞整体趋势与 HCT116 细胞一致，且联合处理组相比于照射组而言显现出更显著的 G2 期阻滞 ($P<0.05$)。这些结果可能由于电离辐射直接对 DNA 损伤，激活 DNA 修复机制阻滞细胞周期进程，cytoD 作用抑制 Cofilin-1 功能诱导细胞 DNA 修复抑制和 G2 期阻滞。

7. cytoD 联合电离辐射对结直肠癌细胞自噬的影响

使用流式细胞术检测各组 HCT116 细胞自噬，6 Gy 照射后 24 h 和 cytoD (1 μ M) 作用 48 h 后，与对照组细胞相比，处理组 HCT116 细胞出现显著的自噬水平增强 ($P<0.05$)，其中药物作用比照射更明显 ($P<0.05$)；与单纯照射相比，cytoD 的作用也使 HT29 细胞的自噬水平显著增强 ($P<0.05$)，但从整体而言，HT29 细胞的自噬水平远低于 HCT116 细胞。电离辐射损伤结直肠癌细胞，激活修复和自噬进程清除细胞损伤，而 cytoD 对结直肠癌细胞的损伤尤其是细胞骨架和带膜细胞器更加显著。

8. cytoD 联合电离辐射对结直肠癌细胞凋亡的影响

6 Gy 照射后 24 h 和 cytoD (1 μ M) 作用 48 h 后，使用流式细胞术检测各组细胞凋亡。其中 HCT116 细胞与对照组细胞相比，照射后细胞凋亡水平明显增强 ($P<0.05$)，但加药组细胞凋亡水平更显著 ($P<0.05$)，联合处理组也显现出高凋亡水平 ($P<0.05$)，电离辐射损伤细胞 DNA 诱导了 HCT116 细胞凋亡，HCT116 因具有一定的辐射抗性而表现出低量升高 ($P<0.05$)；而 cytoD 对 HCT116 细胞的毒性较大，从细胞骨架和线粒体膜损伤到 DNA 修复抑制，都能增强 HCT116 细胞的凋亡水平。在相同处理条件下的 HT29 细胞，相对于对照组细胞而言，电离辐射同样诱导细胞凋亡水平上升，但 cytoD 的作用抑制了 HT29 细胞的凋亡；

联合处理组中细胞凋亡水平也显著低于照射组 ($P<0.05$)，即 cytoD 的作用反而抑制了电离辐射诱导的 HT29 细胞凋亡。

结论：

1. CytoD 联合电离辐射可以促进人结直肠癌细胞内 Cofilin-1 磷酸化，且降低 HCT116 细胞内 Cofilin-1 蛋白表达；
2. CytoD 联合电离辐射可以破坏人结直肠癌细胞骨架的完整性；
3. CytoD 通过对 Cofilin-1 活性的抑制作用，从而诱导辐照后人结直肠癌细胞的细胞增殖抑制、DNA 损伤、线粒体损伤，G2 期阻滞与自噬。

总的来说，cytoD 作为代表性肌动蛋白聚合抑制剂，能有效抑制肿瘤细胞的增殖并增强辐射所致的细胞损伤，但对人体正常组织细胞的损害程度和是否对所有的肿瘤细胞有杀伤效应还有待于研究。

关键词：

电离辐射，结直肠癌，Cofilin-1，线粒体

Abstract

Effect of Cofilin-1 on Radiation Response of Human Colorectal Cancer cells

Colorectal Cancer (CRC), as one of the most common cancers in our country, ranks the forefront of malignant tumors in terms of incidence and mortality. In addition to genetic factors, CRC is also influenced by lifestyle (such as smoking, drinking, sedentary, etc.) and dietary habits (high fat diet, lack of dietary fiber, etc.). In recent years, the social development has led to changes in our national life and work schedule, which has gradually shifted the incidence of CRC from elderly patients over 50 years old to young and middle-aged groups. Early CRC can be surgically resected directly, but most patients are already in the middle and late stage when CRC is diagnosed, which is prone to peripheral tissue infiltration and distant metastasis. Therefore, radiotherapy is often combined with preoperative and postoperative irradiation to improve curative effect, dissection of peripheral lymph nodes and palliative treatment in the late stage. However, due to the high radiation resistance of CRC, the effect of radiotherapy alone is decreased, which seriously affects the prognosis of radiotherapy patients.

As a member of the ADF/Cofilin family, Cofilin-1 can specifically bind actin filaments and cleave to produce G-actin to regulate actin dynamics, thereby participating in the regulation of cells by remodeling the cytoskeleton. As one of the most widely present cellular components in eukaryotic cells, the cytoskeleton is involved in a variety of life activities, such as cell migration, cell proliferation, cycle regulation, apoptosis necrosis, damage repair, etc. It has been reported that Cofilin is abnormally expressed in some human malignant tumor cells, and Cofilin-1 is involved in DNA repair of radiation-resistant cancer cells, thereby affecting the prognosis of radiotherapy in tumor patients. However, the biological effects of radiation in

colorectal cancer cells and the role of Cofilin-1 in it are still not fully understood. Therefore, in this study, human colorectal cancer cells were the main research object, and Cofilin-1 was used as the target protein to explore the regulatory effect and possible mechanism of Cofilin-1 in the radiation response response of human colorectal cancer cells.

Purpose:

Taking Cofilin-1 protein as the research starting point, the role of Cofilin-1 in the radiation biological effect in human colorectal cancer cells was explored through the Cofilin-1 inhibition and regulation of cytochalasin D (hereinafter referred to as cytoD), so as to provide a theoretical basis for optimizing the radiotherapy scheme and clinical diagnosis and treatment of colorectal cancer.

Method:

1. HCT116 and HT29 cells were irradiated with 0.1 Gy (0.0134 Gy/min) and 2, 4, 6, 8 Gy (1.02 Gy/min) doses of X-ray, respectively. Cells were collected 24 h after irradiation, and WB was used to detect the effect of radiation on the expression of Cofilin-1 and its phosphorylated protein.
2. The proliferation ability of colorectal cancer cells after 24 h under the above dose of X-ray radiation was detected by CCK8 method.
3. Using phalloidin staining method to detect changes in the cytoskeleton of colorectal cancer cells.
4. Adoption γ H2AX immunofluorescence assay was used to detect the level of DNA damage in colorectal cancer cells.
5. Detection of ROS production in colorectal cancer cells before and after irradiation using DCFH-DA probe method.
6. Using JC-1 probe method to detect mitochondrial membrane potential in colorectal cancer cells.
7. Cell cycle changes in colorectal cancer were detected by flow cytometry.

8. Flow cytometry was used to detect the autophagy level of colorectal cancer cells.

9. The apoptosis level of colorectal cancer cells was detected by flow cytometry.

Result:

1. Effects of ionizing radiation on proliferation rate and Cofilin-1 expression in colorectal cancer cells

Compared with the control group (0 Gy), the proliferation capacity of HCT116 cells decreased with the increase of dose 24 h after irradiation, and the trend was the most obvious at dose 6 Gy. Compared with the control group of HCT116 cells, the expression of Cofilin-1 decreased after irradiation, the expression of phosphorylated Cofilin-1 increased at large doses (6 and 8 Gy), and the expression of phosphorylated Cofilin-1 increased by about 0.5 times at 6 Gy, the difference was the largest.

Compared with the control group (0 Gy), the proliferation ability of HT29 cells decreased at 2 and 4 Gy compared with HCT116 cells, but the proliferation ability of HT29 cells increased with the increase of dose at 6 and 8 Gy (still lower than that of the control groups in their respective groups). Compared with HT29 cells in the control group, there was no significant difference in Cofilin-1 expression. The expression of phosphorylated Cofilin-1 showed an overall upward trend, and the trend was most pronounced at 6 Gy (the expression increased by about 1 times).

2. Effect of cytoD combined with ionizing radiation on cytoskeleton of colorectal cancer cells

After Phalloidin staining, the boundaries of HCT116 cells in the control group were intact and clear, but there were no obvious skeletal fibrofilaments in the cytoplasm, which may be related to the vigorous proliferation of its tumor cells. 24 h after 6 Gy irradiation can induce stress repair response in HCT116 cells, resulting in stress fibers in stable cell state. cytoD (1 μ M) could significantly destroy the original structure of the cytoskeleton after 48 h, and staining showed the scattered distribution of actin. After the combined treatment, HCT116 cells did not show obvious stress

fibers, and the results showed that cytoD inhibited the function of Cofilin-1 depolymeric actin, so that the skeleton of HCT116 cells could not be repaired in time after receiving irradiation, which may be related to the inability of Cofilin-1 expression reduction cells to clear skeleton breakpoints and provide sufficient amounts of G-actin. HT29 has a small cell diameter compared to HCT116 overall, and no obvious stress fibers can be observed after staining, but the results still show the destructive effect of cytoD on cytoskeletal integrity.

3. Effect of cytoD combined with ionizing radiation on DNA damage in colorectal cancer cells

γ H2AX immunofluorescence staining showed that focal damage occurred in the nucleus of HCT116 cells after 2 h after 6 Gy irradiation and cytoD (1 μ M) for 24 h, and the number of HCT116 cells with focal damage in the nucleus after the combination of the two was significantly increased ($P<0.05$), and the staining results of HT29 cells were consistent with the trend of HCT116 cells, and the effect of cytoD was more obvious than that of irradiation ($P<0.05$). These results indicate that both ionizing radiation and cytoD can lead to colorectal cancer cell DNA damage, and the DNA damage of colorectal cancer cells is exacerbated when the two work together.

4. Effect of cytoD combined with ionizing radiation on MMP depolarization of colorectal cancer cells

Immunofluorescence staining of JC-1 showed that 2 h after 6 Gy irradiation and 24 h after cytoD (1 μ M) action, The fluorescence of some HCT116 cells gradually showed green fluorescence ($P<0.05$) from high-potential red fluorescence, and the combined treatment increased the ratio of green-red fluorescence of HCT116 cells, that is, HCT116 cells showed a stronger MMP depolarization effect ($P<0.05$), and HT29 cells had a similar trend to HCT116 cells, and the dosing group had a lower effect on the MMP depolarization effect than the irradiation group ($P<0.05$). These results may be due to mitochondrial membrane potential depolarization due to ionizing radiation and cytoD damage to the mitochondrial membrane of cells.

5. Effect of cytoD combined with ionizing radiation on ROS accumulation in colorectal cancer cells

The detection of ROS by DCFH-DA probe showed that after 2 h after 6 Gy irradiation and 24 h after cytoD (1 μ M) action, compared with the HCT116 cell control group, some cells showed the green fluorescence signal ($P<0.05$) of ROS, while the green fluorescence signal intensity of cells in the combined treatment group was more obvious ($P<0.05$), and HT29 cells also showed the same trend as HCT116 cells. In addition to the direct production of cellular ROS caused by ionizing radiation, it may also be related to mitochondrial membrane destruction, endogenous ROS release and overall cellular ROS clearance disorders due to cytoD action.

6. Effect of cytoD combined with ionizing radiation on cell cycle of colorectal cancer cells

HCT116 cell cycles were detected by flow cytometry, 24 h after 6 Gy irradiation and 48 h after cytoD (1 μ M) action, compared with control cells, HCT116 cells in the treatment group had significant G2 phase blockade ($P<0.05$), in which the drug effect was more obvious than that of ionizing radiation ($P<0.05$), and the overall trend of HT29 cells was consistent with that of HCT116 cells, and the combined treatment group showed a more significant G2 phase arrest ($P<0.05$) than that in the irradiation group. These results may be due to ionizing radiation of direct damage to DNA, activation of DNA repair mechanisms to block cell cycle progression, cytoD action inhibition of Cofilin-1 function induced cellular DNA repair inhibition and G2 phase arrest.

7. Effect of cytoD combined with ionizing radiation on autophagy of colorectal cancer cells

Autophagy was detected by flow cytometry in each group of HCT116 cells, 24 h after 6 Gy irradiation and 48 h after cytoD (1 μ M) action, compared with control cells. HCT116 cells in the treatment group had a significant increase in autophagy level ($P<0.05$), in which the drug effect was more obvious than that of ionizing radiation

($P<0.05$) , and compared with ionizing radiation alone, the effect of cytoD also significantly enhanced the autophagy level of HT29 cells ($P<0.05$) , but overall, the autophagy level of HT29 cells was much lower than that of HCT116 cells. ionizing radiation injures colorectal cancer cells to activate repair and autophagy processes to clear cell damage, while cytoD damage to colorectal cancer cells, especially cytoskeleton and membrane organelles, is more significant.

8. Effect of cytoD combined with ionizing radiation on apoptosis of colorectal cancer cells

Apoptosis in each group was detected by flow cytometry for 24 h after 6 Gy irradiation and cytoD (1 μ M) for 48 h. Compared with the control group, the apoptosis level of HCT116 cells was significantly enhanced after irradiation ($P<0.05$) , but the apoptosis level of HCT116 cells was more significant ($P<0.05$) in the dosing group, and the combined treatment group also showed a high apoptosis level ($P<0.05$) , ionizing radiation damaged cell DNA induced apoptosis of HCT116 cells, and HCT116 showed a low increase ($P<0.05$) due to certain radiation resistance. CytoD is more toxic to HCT116 cells, from cytoskeletal and mitochondrial membrane damage to DNA repair inhibition, which can enhance the apoptosis level of HCT116 cells. Compared with the control cells, ionizing radiation also induced an increase in apoptosis levels in HT29 cells under the same treatment conditions, but the effect of cytoD inhibited the apoptosis of HT29 cells. The apoptosis level in the combined treatment group was also significantly lower than that in the irradiation group ($P<0.05$) , that is, the effect of cytoD inhibited the apoptosis induced by ionizing radiation.

Conclusion:

1. CytoD combined with ionizing radiation can promote Cofilin-1 phosphorylation in human colorectal cancer cells and reduce the expression of Cofilin-1 protein in HCT116 cells.

2. CytoD combined with ionizing radiation can destroy the integrity of the cytoskeleton of human colorectal cancer cells.

3. CytoD inhibits Cofilin-1 activity, thereby inducing cell proliferation inhibition, DNA damage, mitochondrial damage, G2 phase blockade and autophagy of human colorectal cancer cells after irradiation.

In general, cytoD, as a representative actin polymerization inhibitor, can effectively inhibit the proliferation of tumor cells and enhance radiation-induced cell damage, but the degree of damage to normal tissue cells in the human body and whether it has a killing effect on all tumor cells remains to be studied

Key words:

Ionizing radiation, Colorectal cancer, Cofilin-1, mitochondria

以上内容仅为本文档的试下载部分，为可阅读页数的一半内容。如要下载或阅读全文，请访问：<https://d.book118.com/345313230210011114>