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Attenuation of p53 signaling by CDK8/19 sensitizes tumor cells to ionizing radiation

Kuchur O.A.[1], Zavirskiy A.V.[2], Shtil A.A.[1,3]

1 International Institute 'Solution Chemistry of Advanced Materials and Technologies', ITMO University, 197101, Saint-Petersburg, Russia
2 Kirov Military Medical Academy, 194044 Saint-Petersburg, Russia
3 Blokhin National Medical Research Center of Oncology, 115478 Moscow, Russia

E-mail: kuchur@scamt-itmo.ru

Background:

Photon Beam Radiotherapy is one of the most widespread and effective methods of fighting malignant tumors. However, in response to ionizing radiation, survival mechanisms are activated in tumor cells, which reduces the effectiveness of treatment and increases the likelihood of disease progression. Signaling pathways regulated by the p53 tumor suppressor play a key role in the occurrence of this phenomenon. This mechanism is required to stop DNA replication and start repair programs; in the case of severe stress exposure and large-scale cellular damage, cell death cascades are activated (Fig. 1.)

The regulation of the p53-dependent response of cells to ionizing radiation has at least two aspects: on the one hand, after DNA damage, the level of the p53 protein increases, which causes the cell cycle to stop in the G1 and G2 / M phases and, therefore, makes it possible for DNA repair and accumulation of cells at these checkpoints. Cells with mutant p53 are not arrested after irradiation, resulting in death. On the other hand, cells carrying inactivating p53 mutations can survive - since p53-dependent apoptosis does not function - and maintain the survival of surrounding cells (paracrine regulation of survival), allowing the tumor to recur. One of the probable mechanisms responsible for this phenomenon is the reprogramming of gene transcription with the participation of protein kinases CDK8 / 19. This mechanism ensures the activation of a number of anti-apoptotic genes and the formation of paracrine protection. Thus, the problem of tumor radiosensitivity requires both elucidation of the role of individual p53-dependent cascades in tumor cells and prevention of transcriptional responses of the microenvironment.

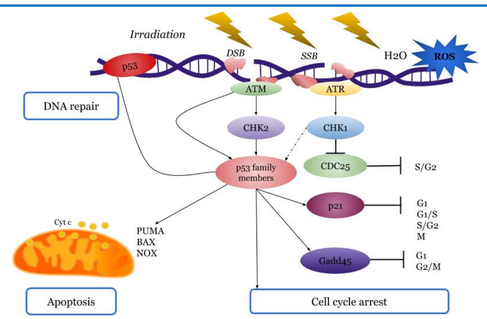

Fig. 1. The mechanisms of the cellular response to ionizing radiation with the participation of the p53 protein

Objective:

To investigate new molecular mechanisms of survival and death of human tumor cells in response to ionizing irradiation with gamma photons and to determine the regulatory role of p53 and reprogramming of gene transcription (protein kinase CDK8 / 19) in this process.

Goals:

1. To study the molecular mechanisms of the response of colorectal cancer cells (HCT116 line) with intact p53 and an isogenic subline with non-functioning p53 (HCT116p53KO) to a single irradiation with gamma photons in a therapeutic dose range of 1-6 Gy: survival, cell cycle disturbances, molecular cascades of death.
2. Establish experimental conditions for the survival of irradiated cells with different p53 status.
3. Explore the possibility of enhancing the death of cells with different p53 status when combined with radiation and inhibition of transcription reprogramming.

Results:

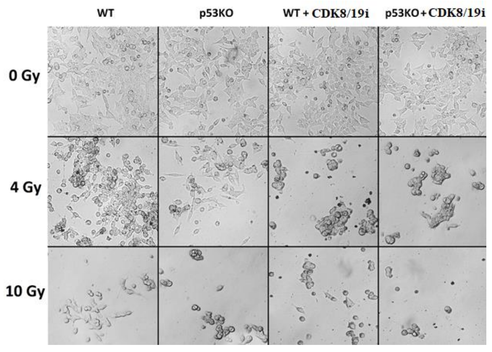

Fig. 2. Photo of HCT116 cells with different p53 status on day 3 after irradiation with a dose of 4 Gy and and addition of the CDK8/19 inhibitor

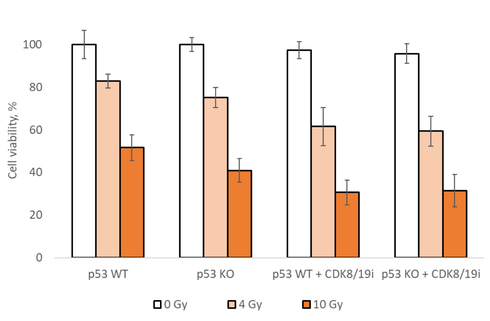

Fig. 3. Survival (%) of HCT116 cells exposed to various doses of gamma radiation ± 1 uM CDK8/19i on day 3 after irradiation

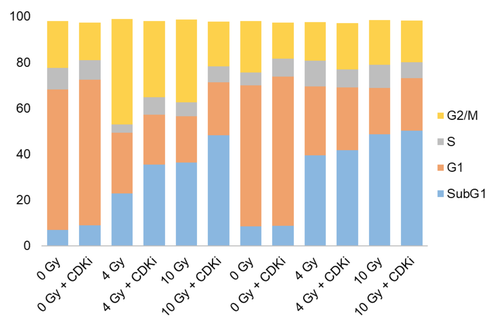

Fig. 4. Distribution of phases in the cell cycle (flow cytometry) of the HCT116 (WT and p53KO) line under irradiation of 0, 4, 10 Gy ± 1 uM CDK8/19i. Day 3 after irradiation. CDKi – CDK8/19 inhibitor

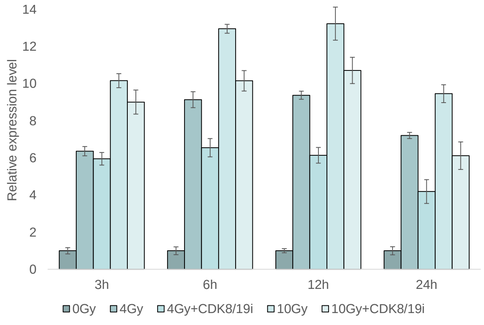

Fig. 5. Expression level of Tp53 gene in HCT116 WT after a combination of irradiation with 4 Gy and CDK8/19 inhibitor. X axis - the time after irradiation, h.

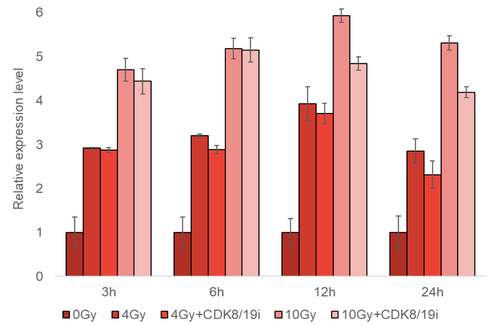

Fig. 6. Expression level of CDKN1A gene in HCT116 WT after a combination of irradiation with 4 Gy and CDK8/19 inhibitor. X axis - the time after irradiation, h.

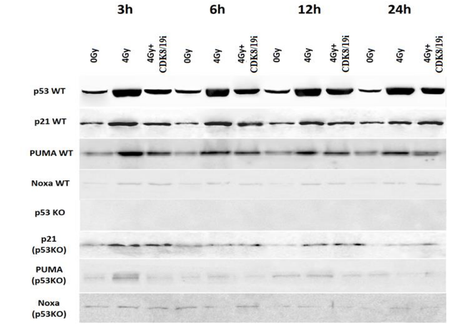

Fig. 7. The amount of p53, p21, PUMA, and Noxa proteins in HCT116 and HCT116p53KO cells after a combination of irradiation with 4 Gy and CDK8/19 inhibitor. Above shows the time after irradiation, h.

Methods:

- HCT116 (p53 wild type) and isogenic HCT116p53KO (p53 knockout) human colorectal carcinoma cells
- CDK8 inhibitor: SnxB (1 uM). Concentrations were chosen as target selective
- Dose of irradiation: 4 Gy γ-photons for long-term and 4-10 Gy for short-term experiments
- Cytotoxicity analysis: MTT and clonogenic assays
- Cell biology methods: flow cytometry, X-Gal (cell senescence) staining
- Molecular biology: immunoblotting, RT-PCR and qPCR

Conclusion:

In human colon carcinoma cells HCT116 with different p53 status (wild type and p53 knockout), we demonstrated that a selective inhibitor of CDK8 / 19 (CDK8 / 19i) in combination with irradiation doses of 4-10 Gray prevents the activation of p53 signaling. By itself, CDK8 / 19i (up to 1 μM) does not affect cell survival for at least 14 days. However, in combination with 4 or 10 Gy, CDK8 / 19i caused the death of tumor cells with a normally functioning p53 gene and had practically no effect on the p53 knockout line (Fig. 2-3). The combination of the inhibitor with gamma-radiation effectively prevented cell accumulation at the G_2 / M checkpoint of the cell cycle and significantly increased the percentage of cells in the apoptotic $SubG_1$ fraction (Fig. 4). These effects have been observed in mass cell culture as well as in clonogenic assays.

Irradiation of HCT116 cells induced p53, p21 at the mRNA level, as well as the proapoptotic PUMA and Noxa at the protein level, while none of these markers were regulated in the HCT116p53KO. The addition of CDK8 / 19i was accompanied by a weakening of mRNA (Fig. 5-6) and proteins p53 and p21, PUMA and Noxa (Fig. 7). In the HCT116p53KO subline, the CDK8 / 19i did not affect the radiation effects. These results suggest the involvement of p53-mediated processes, especially p53 / p21-dependent cell cycle arrest, in the escape of tumor cells from radiation-induced death.

Our results show that inhibition of CDK8 / 19 in irradiated wild-type p53 cells is functionally similar to p53 knockout: pharmacological attenuation of p53 responses (HCT116 cells) or genetic inactivation of p53 (HCT116p53KO cells) abolish the G_2 / M checkpoint and prevent radiation-induced damage repair. Thus, inhibition of CDK8 / 19-mediated transcriptional reprogramming is a therapeutically attractive approach to tumor radiosensitization.

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