ISSN 2618-1584 Vol. 7, Issue 4: 15-21, DOI: 10.25236/FMSR.2025.070402

The radiobiological mechanism of FLASH radiotherapy

Lingbo Zhang[a], Meifeng Fu[b,*]

Department of Oncology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Hubei, Wuhan, China
[a]956588504@qq.com, [b]fmf15778378@qq.com
**Corresponding author*

Abstract: *The core goal of radiotherapy is to eradicate tumors and minimize toxicity to normal tissues to the greatest extent. FLASH radiotherapy (Flash-RT), as a cutting-edge technology in the field of tumor radiotherapy, is regarded as an important direction to break through the limitations of the traditional treatment window. Recent preclinical studies have shown that FLASH-RT significantly expands the therapeutic window of radiotherapy through its unique characteristics of extremely short irradiation time (<0.1 seconds) and ultra-high dose rate (>40 Gy/s) - it can not only enhance the anti-tumor effect and maintain the tumor control probability (TCP) by increasing the dose, It can also reduce the probability of normal tissue complications (NTCP). Furthermore, the significant reduction in its treatment time can avoid the positioning errors caused by organ movement and expand the group of treatable patients. However, due to the extremely short action time of FLASH-RT, the current mechanism research on its biological effects is mostly based on indirect evidence, and some mechanisms are not yet clear and there are contradictory conclusions. This article systematically reviews the radiobiological mechanism of FLASH-RT and, in combination with the limitations of existing research, proposes the key directions for future research.*

Keywords: *FLASH, Biological mechanism, Tumor*

1. Introduction

Cancer is one of the main causes of human death [1]. In 1895, Rontgen discovered X-rays, and numerous studies subsequently revealed the potential of radiotherapy in eradicating tumors[2]. As one of the core treatment methods for cancer, radiotherapy can prolong the overall survival time of cancer patients[3], improve their quality of life [4], and even achieve radical cure of tumors [5]. Early studies have shown that high-dose radiation kills tumor cells while being toxic to normal tissues. Therefore, the dose of radiotherapy needs to take into account both the tumor-killing effect and the avoidance of toxicity to normal tissues. However, the overall anti-tumor efficacy of radiotherapy still has limitations [6]. The treatment window is defined as the dose area between the probability of complications in normal tissues (NTCP) and the probability of tumor control (TCP) curves. Expanding this window is the core goal of radiotherapy.

The standard dose rate during radiotherapy is within the range of 0.5-20 Gy/min. As early as the 1960s, the radiotherapy community had proposed the idea of protecting normal tissues by increasing the dose rate. Dewey and Boag first confirmed that the tolerance of mice to radiation at ultra-high dose rates was significantly higher than that at standard dose rates [7]. It is worth noting that the radiation resistance of bacteria at ultra-high dose rates is similar to that under hypoxic conditions, and cells have the strongest radiation tolerance in an hypoxic environment. Dose limitation of organs at risk around the tumor often leads to insufficient dose in the target area, which may be a key factor restricting the efficacy of anti-tumor treatment. Although modern techniques such as volumetric modulated arc therapy (VMAT), Tomotherapy and proton radiotherapy can optimize dose distribution [8,9] and reduce toxicity in normal tissues, the improvement of anti-tumor effects is still relatively limited [10]. FLASH radiotherapy (Flash-RT) is a new type of radiotherapy technique based on an ultra-high dose rate (≥40 Gy/s). Compared with conventional radiotherapy (CONV-RT), Flash-RT can significantly reduce the toxicity of normal tissues while maintaining the anti-tumor effect, and this phenomenon is defined as the FLASH effect [11]. Preclinical studies have shown that FLASH - RT can effectively reduce the lung [12,13] ,intestines [14,15], brain[16]and skin toxicity[17], while retaining the antitumor activity[13,18,19]. In view of the above breakthrough

ISSN 2618-1584 Vol. 7, Issue 4: 15-21, DOI: 10.25236/FMSR.2025.070402

discoveries, FLASH-RT is regarded as a revolutionary technology in the field of radiotherapy [20]. However, due to the extremely short irradiation time of FLASH-RT (<0.1 seconds), the study of its effect mechanism still relies on indirect evidence, and some biological mechanisms remain unclear.

2. The biological mechanism of flash

The radiobiological mechanism of FLASH radiotherapy is significantly complex and has not been fully clarified at present. Existing studies have shown that its effect mechanism not only involves the regulatory effects on stem cells and immune cells, but also covers the protective effect on the vascular system and other potential biological pathways.

2.1 The influence of FLASH radiotherapy on stem cells

The reduction of stem cell senescence may play a key role in the normal tissue protective effect of FLASH-RT. Unlike apoptosis, senescent cells secrete pro-fibrotic cytokines (such as IL-6, TGF-β, IL-1α), thereby inducing pulmonary fibrosis [21,22]. In addition, stem cell senescence can hinder the cell regeneration process after radiation damage[21,23].

Wanyi Tang et al. successfully induced the directed differentiation of stem cells and activated single-target stem cells in vivo and in vitro by applying 100 milliseconds FLASH-RT to the submicron cytoplasmic region of primary adipose-derived stem cells [22]. Fouillade [23] et al. conducted a preclinical study using C57BL/6J mice to compare the effect differences between FLASH-RT (>40 Gy/s, 17 Gy) and CONV-RT (<0.003 Gy/s, 17 Gy). It was found that the lung injury in the FLASH-RT group was significantly alleviated and the anti-tumor effect was comparable. Further mechanism studies have shown that the lung-protective effect of FLASH-RT is related to the retention of stem cell replication ability - the number of senescent stem cells (with reduced or lost replication ability) in this group is 50% less than that in the CONV-RT group. It is worth noting that in TERC^{-/-} mice (with extremely short telomeres, simulating the aging model of stem cells), the lung-protective effect of Flash-RT disappeared, suggesting that the aging state of stem cells is an important prerequisite for the FLASH effect[23].

In the field of tumor stem cell research, Marzia Mare et al. systematically summarized the predictive value of tumor stem cell markers for the radiotherapy response of rectal cancer[24]. Yoon G et al. found that the burden of tumor stem cells was significantly correlated with the residual lesions after preoperative chemoradiotherapy for rectal cancer [25]. Chen T[26] et al confirmed that the chromatin structure of colon cancer stem cells could affect their radiosensitivity, while Chen Q et al [27] found that Polydatin enhanced radiosensitivity by inducing apoptosis of colorectal cancer stem cells. Anuja K et al. irradiated parental HCT116/HCT-15 cells and their derived colonic bulbs, combined with cell survival tests and cycle analyses, and found that radiation could induce DNA damage responses and drug resistance in colorectal cancer stem cells through pathways such as γ-H2AX focus formation and ATM phosphorylation. At the same time, there were abnormal expressions of CSC markers (CD44, KLF4) and telomere components (TRF2, RAP1, hTERT) [28]. Puglisi C et al. further pointed out that the radiosensitivity of tumor stem cells can be used as a potential predictive indicator for the radiotherapy response of individuals with locally advanced rectal cancer[29].

The in vitro study by Yang et al. showed that FLASH-RT (109 Gy/s, 6-9 Gy) could induce apoptosis, pyroptosis and necrosis of tumor stem cells and normal tumor cells. However, the former had a stronger ability to resist radiation, which might be related to the enhanced autophagy mediated by lysosomes. It is notable that this study did not directly compare the differences in damage to tumor stem cells between FLASH-RT and CONV-RT, and its specific role in the overall anti-tumor effect of FLASH-RT still needs to be further verified [30].

2.2 The influence of FLASH radiotherapy on immunity

Radiation damage is essentially an aseptic inflammatory process, in which immune function plays a key role [31,32]. Transforming growth factor -β (TGF-β), as an important inflammatory factor, not only participates in DNA damage repair, but also promotes radiation-induced pulmonary fibrosis by activating cellular inflammatory pathways [33]. Multiple studies have shown that the expression level of TGF-β in the FLASH-RT group was significantly lower than that in the CONV-RT group [12,34,35]. Fouillade et al. [23] utilized animal models and radiotherapy parameters consistent with the previous text (C57BL/6J mice, FLASH-RT: >40 Gy/s, 17 Gy;) CONV-RT: <0.003 Gy/s, 17 Gy). It was found that the upregulation amplitudes of the pro-inflammatory gene EGR1 and inflammatory factors (TGF-β1, NF-κB) in the

ISSN 2618-1584 Vol. 7, Issue 4: 15-21, DOI: 10.25236/FMSR.2025.070402

FLASH-RT group were significantly lower than those in the control group, suggesting its inhibitory effect on the immune inflammatory response.

Zhu [36]et al. used X-ray FLASH-RT (>150 Gy/s, 10 Gy/15 Gy) in the intestinal model of BALB/c mice and confirmed that it could reduce intestinal toxicity and decrease the levels of inflammatory blood cells (white blood cells, lymphocytes, neutrophils) and pro-inflammatory cytokines (TNF-α, IL-6). Meanwhile, it inhibits lipid peroxidation reactions. Preclinical studies have shown that microglial activation-mediated chronic neuritis is closely related to radiation-induced brain injury [37,38]. In the study of brain injury, Montay-Gruel et al[39]discovered through electron beam FLASH-RT (5.6×10^6 Gy/s, 10 Gy) that The expressions of astrocyte activation markers (GFAP, TLR4) in the FLASH-RT group were significantly lower than those in the CONV-RT group (0.1 Gy/s, 10 Gy). Dokic et al[40] further confirmed that it could reduce chronic neuroinflammation mediated by microglia/macrophages.

In the field of tumor immunology, the [41-43]series of studies by the Saigusa S team have shown that the expressions of stem cell markers such as CD133, OCT4, and SOX2 are associated with distant recurrence and radiotherapy resistance after chemoradiotherapy for rectal cancer, while the levels of LGR5 and CD44 can predict the preoperative chemoradiotherapy efficacy of locally advanced rectal cancer. Luo CW et al[44] found that G9a regulates the DNA damage response and chemoradiotherapy resistance of colon cancer stem cells through the PP2A-RPA axis.

Existing studies [45-47] have shown that circulating immune cells play a key role in the repair of normal tissues and anti-tumor processes after radiotherapy. Based on this, the academic community speculates that the protective effect of Flash-RT (FLASH therapy) on circulating immune cells is very likely to be one of the important mechanisms of its unique FLASH effect. Jin et al[48] conducted a comparative study on the effects of FLASH-RT and traditional fractional radiotherapy (cvn-rt) on circulating immune cells by means of computer simulation technology. The results showed that the killing rate of FLASH-RT on circulating immune cells was only 5%-10%, significantly lower than 90%-100% of cvn-rt. However, since this research only remains at the level of computer simulation, the reliability of its conclusion urgently needs to be further verified through experiments. Furthermore, this study only focused on circulating immune cells and did not evaluate immune cells in immune organs and tumor tissues. Therefore, the protective effect of FLASH-RT on overall immune function still needs to be further explored.

Eggold et al[49] systematically evaluated the effect of FLASH-RT on tumor immune cells by constructing an animal model of peritoneal ovarian cancer. Under the condition of the same dose (14Gy), after treatment with FLASH-RT (210 Gy/s) and COVN-RT (0.126 Gy/s), both showed a decrease in regulatory T cells and an increase in CD8+ T cells in the tumor tissues. It is notable that when radiotherapy is combined with PD-1 inhibitors, the FLASH-RT group shows better anti-tumor effects than the cvn-rt group. Although this research result still needs to be supported by more preclinical studies, it has fully demonstrated the broad application prospects of FLASH-RT combined with immunotherapy[49].

The importance of immune function in maintaining the anti-tumor effect of FLASH-RT has also attracted much attention. Liljedahl et al[19]took tumor-bearing mice as the research subjects and compared the anti-tumor effects of FLASH-RT (66 Gy/s, 8Gy×2 and 12.5 Gy×2 groups) and COVN-RT (0.133Gy/s, 8Gy×2 and 12.5 Gy×2 groups). And through conducting a secondary challenge experiment on the cured mice after radiotherapy, their long-term anti-tumor ability was evaluated. The research found that there was no significant difference in the anti-tumor effect between the two radiotherapy methods (median survival time: 100 days vs 100 days, $p < 0.05$), among which the FLASH-RT group cured 8 mice and the cvn-rt group cured 6 mice. This discovery provides a new research direction for the subsequent in-depth exploration of the synergistic mechanism between FLASH-RT and immune function.

Vascular injury caused by radiotherapy is regarded as an important component of radiation injury [50,51]. Favaudon et al[12] found that compared with CONV-RT, FLASH-RT could significantly reduce the acute apoptosis of bronchial vascular endothelial cells. Two studies on brain injury further indicated that FLASH-RT was significantly superior to CONV-RT in maintaining the integrity of brain microvascular structures, which might be related to its protective effect on cognitive function. However, the existing evidence can only confirm that the degree of vascular damage caused by FLASH-RT is lower than that of CONV-RT, and its specific influence mechanism on upstream regulatory genes still needs to be further explored [12,40,52].

2.3 Other possible biological mechanisms

Three preclinical studies have shown that the protective effect of FLASH-RT on the intestine may be

ISSN 2618-1584 Vol. 7, Issue 4: 15-21, DOI: 10.25236/FMSR.2025.070402

related to the protective effect of FLASH-RT on intestinal crypt cells [53-55]. Ruan et al[55] also found that the effect of FLASH-RT on the intestinal flora is less than that of COVN-RT, which may be more conducive to the protection of intestinal function. Guo et al[56]found that FLASH-RT could reduce mitochondrial damage mediated by Dynamin-1-like proteins. Jay et al[57] believe that FLASH-RT can generate an early transient strong acid environment, which may be one of the mechanisms by which FLASH-RT protects normal tissues. Ohsawa et al. 58 investigated the effects of proton FLASH-RT (40 Gy/s) and COVN-RT (0.05 Gy/s) on DNA damage. They found that compared with COVN-RT, the single-stranded DNA breaks in the FLASH-RT group were significantly reduced, but the double-stranded DNA breaks were similar. Ohsawa et al[58] speculated that FLASH-RT might effectively reduce non-fatal damages, such as cellular senescence, genomic instability and cell transformation.

3. Conclusion

FLASH radiotherapy has expanded the therapeutic window of radiotherapy through multi-dimensional mechanisms (stem cell protection, immune regulation, and vascular protection), demonstrating significant preclinical advantages. However, its mechanism complexity and technical challenges still require in-depth study.

At the stem cell level, FLASH radiotherapy can reduce the aging of normal stem cells (such as a 50% reduction in the number of senescent stem cells in lung tissue), retain their replication ability, and thereby alleviate radiation damage (such as pulmonary fibrosis). The TERC mice (simulating stem cell aging) experiment showed that the lung-protective effect of FLASH disappeared, further verifying the key role of stem cell aging in it. The resistance of tumor stem cells to FLASH radiotherapy may be related to lysosome-mediated autophagy. However, existing studies have not directly compared the damage differences of FLASH and traditional radiotherapy to tumor stem cells, and its impact on the overall therapeutic effect remains unclear.

At the stem cell level, FLASH radiotherapy can reduce the aging of normal stem cells (such as a 50% reduction in the number of senescent stem cells in lung tissue), retain their replication ability, and thereby alleviate radiation damage (such as pulmonary fibrosis). The TERC mice (simulating stem cell aging) experiment showed that the lung-protective effect of FLASH disappeared, further verifying the key role of stem cell aging in it. The resistance of tumor stem cells to FLASH radiotherapy may be related to lysosome-mediated autophagy. However, existing studies have not directly compared the damage differences of FLASH and traditional radiotherapy to tumor stem cells, and its impact on the overall therapeutic effect remains unclear.

At the level of immune regulation, FLASH radiotherapy significantly reduces the expression of pro-inflammatory factors (such as TGF-β, NF-κB, TNF-α, IL-6), and alleviates the inflammatory responses in tissues such as the intestine and brain (such as the decreased expression of astrocyte activation markers GFAP and TLR4 in the brain). Computer simulations show that the killing rate of circulating immune cells by FLASH (5%-10%) is much lower than that of traditional radiotherapy (90%-100%), and the combination with PD-1 inhibitors can enhance the anti-tumor effect, suggesting that the preservation of immune function may be part of the mechanism of the FLASH effect.

In the blood vessel protection and other mechanisms, FLASH radiation therapy can decrease bronchial vascular endothelial cell apoptosis, protect brain microvascular integrity, thereby reducing the blood vessel damage induced by radiation, may be associated with cognitive function protection. FLASH causes less damage to intestinal crypt cells and intestinal flora, which may be related to its protection of the local microenvironment. FLASH can reduce mitochondrial damage mediated by Dynamin-1-like proteins, and the number of single-stranded DNA breaks is less than that of traditional radiotherapy, but the double-stranded breaks are similar, suggesting that it may play a protective role by reducing non-fatal damage (such as cellular senescence). FLASH may reduce normal tissue damage by inducing an early transient strong acid environment, but this mechanism needs to be further verified.

In general, the future of tumor treatment requires interdisciplinary collaboration, a robust drive mechanism, clinical transformation, and technology standardization, which will together serve as important breakthroughs in the field. With the coordinated development of precise radiotherapy technology and immunotherapy, FLASH radiotherapy is expected to gradually enter the clinical routine within the next 5 to 10 years, providing a new treatment option that is efficient and low-toxic for a large number of cancer patients.

ISSN 2618-1584 Vol. 7, Issue 4: 15-21, DOI: 10.25236/FMSR.2025.070402

References

[1] Schwartz, S.M. (2024). Epidemiology of Cancer. Clin Chem 70, 140-149. 10.1093/clinchem/hvad202.
[2] Thariat, J., Hannoun-Levi, J.M., Sun Myint, A., Vuong, T., and Gérard, J.P. (2013). Past, present, and future of radiotherapy for the benefit of patients. Nat Rev Clin Oncol 10, 52-60. 10.1038/nrclinonc.2012.203.
[3] Kerkmeijer, L.G.W., Groen, V.H., Pos, F.J., Haustermans, K., Monninkhof, E.M., Smeenk, R.J., Kunze-Busch, M., de Boer, J.C.J., van der Voort van Zijp, J., van Vulpen, M., et al. (2021). Focal Boost to the Intraprostatic Tumor in External Beam Radiotherapy for Patients With Localized Prostate Cancer: Results From the FLAME Randomized Phase III Trial. J Clin Oncol 39, 787-796. 10.1200/jco.20.02873.
[4] Rim, C.H., Park, S., Yoon, W.S., Shin, I.S., and Park, H.C. (2023). Radiotherapy for bone metastases of hepatocellular carcinoma: a hybrid systematic review with meta-analyses. Int J Radiat Biol 99, 419-430. 10.1080/09553002.2022.2094020.
[5] Zhang, Z., Liu, X., Chen, D., and Yu, J. (2022). Radiotherapy combined with immunotherapy: the dawn of cancer treatment. Signal Transduct Target Ther 7, 258. 10.1038/s41392-022-01102-y.
[6] Delaney, G., Jacob, S., Featherstone, C., and Barton, M. (2005). The role of radiotherapy in cancer treatment: estimating optimal utilization from a review of evidence-based clinical guidelines. Cancer 104, 1129-1137. 10.1002/cncr.21324.
[7] Dewey, D.L., and Boag, J.W. (1959). Modification of the oxygen effect when bacteria are given large pulses of radiation. Nature 183, 1450-1451. 10.1038/1831450a0.
[8] Cheng, Y.K., Kuo, S.H., Yen, H.H., Wu, J.H., Chen, Y.C., and Huang, M.Y. (2022). The prognostic significance of pretreatment squamous cell carcinoma antigen levels in cervical cancer patients treated by concurrent chemoradiation therapy and a comparison of dosimetric outcomes and clinical toxicities between tomotherapy and volumetric modulated arc therapy. Radiat Oncol 17, 91. 10.1186/s13014-022-02063-w.
[9] Paganetti, H., Botas, P., Sharp, G.C., and Winey, B. (2021). Adaptive proton therapy. Phys Med Biol 66. 10.1088/1361-6560/ac344f.
[10] Lin, S.H., Hobbs, B.P., Verma, V., Tidwell, R.S., Smith, G.L., Lei, X., Corsini, E.M., Mok, I., Wei, X., Yao, L., et al. (2020). Randomized Phase IIB Trial of Proton Beam Therapy Versus Intensity-Modulated Radiation Therapy for Locally Advanced Esophageal Cancer. J Clin Oncol 38, 1569-1579. 10.1200/jco.19.02503.
[11] Favaudon, V., Labarbe, R., and Limoli, C.L. (2022). Model studies of the role of oxygen in the FLASH effect. Med Phys 49, 2068-2081. 10.1002/mp.15129.
[12] Favaudon, V., Caplier, L., Monceau, V., Pouzoulet, F., Sayarath, M., Fouillade, C., Poupon, M.F., Brito, I., Hupé, P., Bourhis, J., et al. (2014). Ultrahigh dose-rate FLASH irradiation increases the differential response between normal and tumor tissue in mice. Sci Transl Med 6, 245ra293. 10.1126/scitranslmed.3008973.
[13] Gao, F., Yang, Y., Zhu, H., Wang, J., Xiao, D., Zhou, Z., Dai, T., Zhang, Y., Feng, G., Li, J., et al. (2022). First demonstration of the FLASH effect with ultrahigh dose rate high-energy X-rays. Radiother Oncol 166, 44-50. 10.1016/j.radonc.2021.11.004.
[14] Zhang, Q., Gerweck, L.E., Cascio, E., Gu, L., Yang, Q., Dong, X., Huang, P., Bertolet, A., Nesteruk, K.P., Sung, W., et al. (2023). Absence of Tissue-Sparing Effects in Partial Proton FLASH Irradiation in Murine Intestine. Cancers (Basel) 15. 10.3390/cancers15082269.
[15] Verginadis, II, Velalopoulou, A., Kim, M.M., Kim, K., Paraskevaidis, I., Bell, B., Oliaei Motlagh, S.A., Karaj, A., Banerjee, E., Finesso, G., et al. (2024). FLASH proton reirradiation, with or without hypofractionation, mitigates chronic toxicity in the normal murine intestine, skin, and bone. bioRxiv. 10.1101/2024.07.08.602528.
[16] Jansen, J., Kimbler, A., Drayson, O., Lanz, B., Mosso, J., Grilj, V., Petit, B., Franco-Perez, J., Simon, A., Limoli, C.L., et al. (2025). Ex vivo brain MRI to assess conventional and FLASH brain irradiation effects. Radiother Oncol 208, 110894. 10.1016/j.radonc.2025.110894.
[17] Zhang, Q., Gerweck, L.E., Cascio, E., Yang, Q., Huang, P., Niemierko, A., Bertolet, A., Nesteruk, K.P., McNamara, A., and Schuemann, J. (2023). Proton FLASH effects on mouse skin at different oxygen tensions. Phys Med Biol 68. 10.1088/1361-6560/acb888.
[18] Iturri, L., Bertho, A., Lamirault, C., Brisebard, E., Juchaux, M., Gilbert, C., Espenon, J., Sébrié, C., Jourdain, L., de Marzi, L., et al. (2023). Oxygen supplementation in anesthesia can block FLASH effect and anti-tumor immunity in conventional proton therapy. Commun Med (Lond) 3, 183. 10.1038/s43856-023-00411-9.
[19] Liljedahl, E., Konradsson, E., Gustafsson, E., Jonsson, K.F., Olofsson, J.K., Ceberg, C., and Redebrandt, H.N. (2022). Long-term anti-tumor effects following both conventional radiotherapy and FLASH in fully immunocompetent animals with glioblastoma. Sci Rep 12, 12285. 10.1038/s41598-022-16612-6.
[20] Maxim, P.G., Keall, P., and Cai, J. (2019). FLASH radiotherapy: Newsflash or flash in the pan? Med Phys 46, 4287-4290. 10.1002/mp.13685.

ISSN 2618-1584 Vol. 7, Issue 4: 15-21, DOI: 10.25236/FMSR.2025.070402

[21] Citrin, D.E., Shankavaram, U., Horton, J.A., Shield, W., 3rd, Zhao, S., Asano, H., White, A., Sowers, A., Thetford, A., and Chung, E.J. (2013). Role of type II pneumocyte senescence in radiation-induced lung fibrosis. J Natl Cancer Inst 105, 1474-1484. 10.1093/jnci/djt212.

[22] Tang, W., Wang, H., Zhao, X., Liu, S., Kong, S.K., Ho, A., Chen, T., Feng, H., and He, H. (2022). Stem cell differentiation with consistent lineage commitment induced by a flash of ultrafast-laser activation in vitro and in vivo. Cell Rep 38, 110486. 10.1016/j.celrep.2022.110486.

[23] Fouillade, C., Curras-Alonso, S., Giuranno, L., Quelennec, E., Heinrich, S., Bonnet-Boissinot, S., Beddok, A., Leboucher, S., Karakurt, H.U., Bohec, M., et al. (2020). FLASH Irradiation Spares Lung Progenitor Cells and Limits the Incidence of Radio-induced Senescence. Clin Cancer Res 26, 1497-1506. 10.1158/1078-0432.Ccr-19-1440.

[24] Mare, M., Colarossi, L., Veschi, V., Turdo, A., Giuffrida, D., Memeo, L., Stassi, G., and Colarossi, C. (2021). Cancer Stem Cell Biomarkers Predictive of Radiotherapy Response in Rectal Cancer: A Systematic Review. Genes (Basel) 12. 10.3390/genes12101502.

[25] Yoon, G., Kim, S.M., Kim, H.J., and Seo, A.N. (2016). Clinical influence of cancer stem cells on residual disease after preoperative chemoradiotherapy for rectal cancer. Tumour Biol 37, 3571-3580. 10.1007/s13277-015-4201-9.

[26] Chen, T., Zhang, Y., Guo, W.H., Meng, M.B., Mo, X.M., and Lu, Y. (2010). Effects of heterochromatin in colorectal cancer stem cells on radiosensitivity. Chin J Cancer 29, 270-276. 10.5732/cjc.009.10694.

[27] Chen, Q., Zeng, Y.N., Zhang, K., Zhao, Y., Wu, Y.Y., Li, G., Cheng, H.Y., Zhang, M., Lai, F., Wang, J.B., and Cui, F.M. (2019). Polydatin Increases Radiosensitivity by Inducing Apoptosis of Stem Cells in Colorectal Cancer. Int J Biol Sci 15, 430-440. 10.7150/ijbs.27050.

[28] Anuja, K., Chowdhury, A.R., Saha, A., Roy, S., Rath, A.K., Kar, M., and Banerjee, B. (2019). Radiation-induced DNA damage response and resistance in colorectal cancer stem-like cells. Int J Radiat Biol 95, 667-679. 10.1080/09553002.2019.1580401.

[29] Puglisi, C., Giuffrida, R., Borzì, G., Di Mattia, P., Costa, A., Colarossi, C., Deiana, E., Picardo, M.C., Colarossi, L., Mare, M., et al. (2020). Radiosensitivity of Cancer Stem Cells Has Potential Predictive Value for Individual Responses to Radiotherapy in Locally Advanced Rectal Cancer. Cancers (Basel) 12. 10.3390/cancers12123672.

[30] Yang, G., Lu, C., Mei, Z., Sun, X., Han, J., Qian, J., Liang, Y., Pan, Z., Kong, D., Xu, S., et al. (2021). Association of Cancer Stem Cell Radio-Resistance Under Ultra-High Dose Rate FLASH Irradiation With Lysosome-Mediated Autophagy. Front Cell Dev Biol 9, 672693. 10.3389/fcell.2021.672693.

[31] Yahyapour, R., Amini, P., Rezapour, S., Cheki, M., Rezaeyan, A., Farhood, B., Shabeeb, D., Musa, A.E., Fallah, H., and Najafi, M. (2018). Radiation-induced inflammation and autoimmune diseases. Mil Med Res 5, 9. 10.1186/s40779-018-0156-7.

[32] Najafi, M., Motevaseli, E., Shirazi, A., Geraily, G., Rezaeyan, A., Norouzi, F., Rezapoor, S., and Abdollahi, H. (2018). Mechanisms of inflammatory responses to radiation and normal tissues toxicity: clinical implications. Int J Radiat Biol 94, 335-356. 10.1080/09553002.2018.1440092.

[33] Wang, J., Xu, Z., Wang, Z., Du, G., and Lun, L. (2021). TGF-beta signaling in cancer radiotherapy. Cytokine 148, 155709. 10.1016/j.cyto.2021.155709.

[34] Buonanno, M., Grilj, V., and Brenner, D.J. (2019). Biological effects in normal cells exposed to FLASH dose rate protons. Radiother Oncol 139, 51-55. 10.1016/j.radonc.2019.02.009.

[35] Velalopoulou, A., Karagounis, I.V., Cramer, G.M., Kim, M.M., Skoufos, G., Goia, D., Hagan, S., Verginadis, II, Shoniyozov, K., Chiango, J., et al. (2021). FLASH Proton Radiotherapy Spares Normal Epithelial and Mesenchymal Tissues While Preserving Sarcoma Response. Cancer Res 81, 4808-4821. 10.1158/0008-5472.Can-21-1500.

[36] Zhu, H., Xie, D., Yang, Y., Huang, S., Gao, X., Peng, Y., Wang, B., Wang, J., Xiao, D., Wu, D., et al. (2022). Radioprotective effect of X-ray abdominal FLASH irradiation: Adaptation to oxidative damage and inflammatory response may be benefiting factors. Med Phys 49, 4812-4822. 10.1002/mp.15680.

[37] Acharya, M.M., Baulch, J.E., Lusardi, T.A., Allen, B.D., Chmielewski, N.N., Baddour, A.A., Limoli, C.L., and Boison, D. (2016). Adenosine Kinase Inhibition Protects against Cranial Radiation-Induced Cognitive Dysfunction. Front Mol Neurosci 9, 42. 10.3389/fnmol.2016.00042.

[38] Zhou, H., Liu, Z., Liu, J., Wang, J., Zhou, D., Zhao, Z., Xiao, S., Tao, E., and Suo, W.Z. (2011). Fractionated radiation-induced acute encephalopathy in a young rat model: cognitive dysfunction and histologic findings. AJNR Am J Neuroradiol 32, 1795-1800. 10.3174/ajnr.A2643.

[39] Montay-Gruel, P., Markarian, M., Allen, B.D., Baddour, J.D., Giedzinski, E., Jorge, P.G., Petit, B., Bailat, C., Vozenin, M.C., Limoli, C., and Acharya, M.M. (2020). Ultra-High-Dose-Rate FLASH Irradiation Limits Reactive Gliosis in the Brain. Radiat Res 194, 636-645. 10.1667/rade-20-00067.1.

[40] Dokic, I., Meister, S., Bojcevski, J., Tessonnier, T., Walsh, D., Knoll, M., Mein, S., Tang, Z., Vogelbacher, L., Rittmueller, C., et al. (2022). Neuroprotective Effects of Ultra-High Dose Rate FLASH Bragg Peak Proton Irradiation. Int J Radiat Oncol Biol Phys 113, 614-623. 10.1016/j.ijrobp.2022.02.020.

[41] Saigusa, S., Inoue, Y., Tanaka, K., Toiyama, Y., Matsushita, K., Kawamura, M., Okugawa, Y., Hiro, J., Uchida, K., Mohri, Y., and Kusunoki, M. (2012). Clinical significance of LGR5 and CD44 expression in locally advanced rectal cancer after preoperative chemoradiotherapy. Int J Oncol 41, 1643-1652.

ISSN 2618-1584 Vol. 7, Issue 4: 15-21, DOI: 10.25236/FMSR.2025.070402

10.3892/ijo.2012.1598.
[42] Saigusa, S., Tanaka, K., Toiyama, Y., Yokoe, T., Okugawa, Y., Kawamoto, A., Yasuda, H., Morimoto, Y., Fujikawa, H., Inoue, Y., et al. (2010). Immunohistochemical features of CD133 expression: association with resistance to chemoradiotherapy in rectal cancer. Oncol Rep 24, 345-350. 10.3892/or_00000865.
[43] Saigusa, S., Tanaka, K., Toiyama, Y., Yokoe, T., Okugawa, Y., Ioue, Y., Miki, C., and Kusunoki, M. (2009). Correlation of CD133, OCT4, and SOX2 in rectal cancer and their association with distant recurrence after chemoradiotherapy. Ann Surg Oncol 16, 3488-3498. 10.1245/s10434-009-0617-z.
[44] Luo, C.W., Wang, J.Y., Hung, W.C., Peng, G., Tsai, Y.L., Chang, T.M., Chai, C.Y., Lin, C.H., and Pan, M.R. (2017). G9a governs colon cancer stem cell phenotype and chemoradioresistance through PP2A-RPA axis-mediated DNA damage response. Radiother Oncol 124, 395-402. 10.1016/j.radonc.2017.03.002.
[45] Wirsdörfer, F., and Jendrossek, V. (2016). The Role of Lymphocytes in Radiotherapy-Induced Adverse Late Effects in the Lung. Front Immunol 7, 591. 10.3389/fimmu.2016.00591.
[46] Campian, J.L., Ye, X., Brock, M., and Grossman, S.A. (2013). Treatment-related lymphopenia in patients with stage III non-small-cell lung cancer. Cancer Invest 31, 183-188. 10.3109/07357907.2013.767342.
[47] Davuluri, R., Jiang, W., Fang, P., Xu, C., Komaki, R., Gomez, D.R., Welsh, J., Cox, J.D., Crane, C.H., Hsu, C.C., and Lin, S.H. (2017). Lymphocyte Nadir and Esophageal Cancer Survival Outcomes After Chemoradiation Therapy. Int J Radiat Oncol Biol Phys 99, 128-135. 10.1016/j.ijrobp.2017.05.037.
[48] Jin, J.Y., Gu, A., Wang, W., Oleinick, N.L., Machtay, M., and Spring Kong, F.M. (2020). Ultra-high dose rate effect on circulating immune cells: A potential mechanism for FLASH effect? Radiother Oncol 149, 55-62. 10.1016/j.radonc.2020.04.054.
[49] Eggold, J.T., Chow, S., Melemenidis, S., Wang, J., Natarajan, S., Loo, P.E., Manjappa, R., Viswanathan, V., Kidd, E.A., Engleman, E., et al. (2022). Abdominopelvic FLASH Irradiation Improves PD-1 Immune Checkpoint Inhibition in Preclinical Models of Ovarian Cancer. Mol Cancer Ther 21, 371-381. 10.1158/1535-7163.Mct-21-0358.
[50] Fauquette, W., Amourette, C., Dehouck, M.P., and Diserbo, M. (2012). Radiation-induced blood-brain barrier damages: an in vitro study. Brain Res 1433, 114-126. 10.1016/j.brainres.2011.11.022.
[51] Yuan, H., Gaber, M.W., McColgan, T., Naimark, M.D., Kiani, M.F., and Merchant, T.E. (2003). Radiation-induced permeability and leukocyte adhesion in the rat blood-brain barrier: modulation with anti-ICAM-1 antibodies. Brain Res 969, 59-69. 10.1016/s0006-8993(03)02278-9.
[52] Allen, B.D., Acharya, M.M., Montay-Gruel, P., Jorge, P.G., Bailat, C., Petit, B., Vozenin, M.C., and Limoli, C. (2020). Maintenance of Tight Junction Integrity in the Absence of Vascular Dilation in the Brain of Mice Exposed to Ultra-High-Dose-Rate FLASH Irradiation. Radiat Res 194, 625-635. 10.1667/rade-20-00060.1.
[53] Levy, K., Natarajan, S., Wang, J., Chow, S., Eggold, J.T., Loo, P.E., Manjappa, R., Melemenidis, S., Lartey, F.M., Schüler, E., et al. (2020). Abdominal FLASH irradiation reduces radiation-induced gastrointestinal toxicity for the treatment of ovarian cancer in mice. Sci Rep 10, 21600. 10.1038/s41598-020-78017-7.
[54] Kim, M.M., Verginadis, II, Goia, D., Haertter, A., Shoniyozov, K., Zou, W., Maity, A., Busch, T.M., Metz, J.M., Cengel, K.A., et al. (2021). Comparison of FLASH Proton Entrance and the Spread-Out Bragg Peak Dose Regions in the Sparing of Mouse Intestinal Crypts and in a Pancreatic Tumor Model. Cancers (Basel) 13. 10.3390/cancers13164244.
[55] Ruan, J.L., Lee, C., Wouters, S., Tullis, I.D.C., Verslegers, M., Mysara, M., Then, C.K., Smart, S.C., Hill, M.A., Muschel, R.J., et al. (2021). Irradiation at Ultra-High (FLASH) Dose Rates Reduces Acute Normal Tissue Toxicity in the Mouse Gastrointestinal System. Int J Radiat Oncol Biol Phys 111, 1250-1261. 10.1016/j.ijrobp.2021.08.004.
[56] Guo, Z., Buonanno, M., Harken, A., Zhou, G., and Hei, T.K. (2022). Mitochondrial Damage Response and Fate of Normal Cells Exposed to FLASH Irradiation with Protons. Radiat Res 197, 569-582. 10.1667/rade-21-00181.1.
[57] Jay-Gerin, J.P. (2020). Ultra-high dose-rate (FLASH) radiotherapy: Generation of early, transient, strongly acidic spikes in the irradiated tumor environment. Cancer Radiother 24, 332-334. 10.1016/j.canrad.2019.11.004.
[58] Ohsawa, D., Hiroyama, Y., Kobayashi, A., Kusumoto, T., Kitamura, H., Hojo, S., Kodaira, S., and Konishi, T. (2022). DNA strand break induction of aqueous plasmid DNA exposed to 30 MeV protons at ultra-high dose rate. J Radiat Res 63, 255-260. 10.1093/jrr/rrab114.