Tumor response to ultra-high dose rate radiation (FLASH) vs. conventional radiotherapy in C57BL/6 and cGAS-/- mice

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Abstract

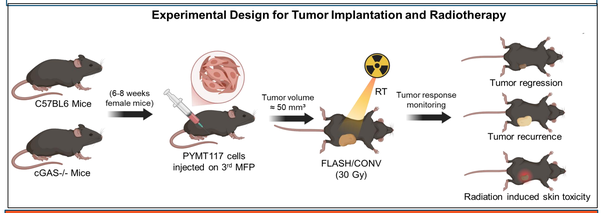
Ultra-high dose rate radiotherapy (FLASH RT) has achieved tumor control comparable to conventional RT (CONV) while markedly reducing normal tissue toxicity. Using an orthotopic breast cancer (BC) model in wild-type (C57BL/6) and cGAS-double knockout (cGAS-/- KO) mice, we compared tumor response and skin toxicity following a single 30 Gy dose of FLASH or CONV RT. Both modalities significantly reduced tumor burden; however, CONV induced severe skin inflammation, whereas FLASH caused only mild lesions. Notably, cGAS-/- mice showed minimal to no detectable toxicity, suggesting that the cGAS-STING pathway may contribute to radiation-induced inflammation. These findings support FLASH RT as a promising, less toxic approach for BC therapy, potentially through modulation of inflammatory responses.

Introduction

Radiotherapy plays a major role in eradicating residual disease in primary BC and metastatic BC. While conventional RT (CONV ≤0.03 Gy/s) is effective, it often causes significant normal tissue toxicity, such as skin inflammation, limiting treatment intensity and patient quality of life[1–3]. FLASH RT is a newer approach delivering ultra-high dose rates (>40 Gy/s), has shown similar tumor control with reduced normal tissue damage (the "FLASH effect")[4, 5]. However, the mechanism by which FLASH spares normal tissue and suppresses tumor growth remains poorly understood. Ionizing radiation activates the cGAS-STING pathway through DNA damage, triggering proinflammatory cytokine production and tissue inflammation[6]. Given that our findings suggest FLASH RT reduces inflammatory toxicity, we investigated the role of the cGAS-STING pathway in mediating responses to FLASH and CONV RT using wild-type and cGAS-/- mice, to better understand how host cGAS signaling contributes to the FLASH effect and tumor control.

Methods and Materials

- PYMT117 BC cells, injected into the 3rd mammary fat pad of 6–8-week-old female C57BL/6 and cGAS-/- mice.
- Once tumors reached ~50 mm³, mice received a single 30 Gy dose to the tumor site using a TrueBeam electron linac.
- FLASH was delivered with 12.8 MeV electrons (>100 Gy/s, mean ~180 Gy/s; pulse width 3.6 µs, 180 Hz, ~1 Gy/pulse, 30 pulses = 30 Gy). Conventional RT used 11.9 MeV electrons (~0.1 Gy/s). Dosimetry was verified using EBT-XD film and an ionization chamber.
- Tumor growth was monitored every other day with callipers, and skin toxicity (inflammation, cracking) was monitored. Euthanasia was performed based on tumor burden or skin damage.
- Tumor volumes were analyzed by two-way ANOVA followed by Tukey's post hoc test; Skin toxicity was graded as 0-5 and $p < 0.05$ was considered significant.



Results

- Both FLASH and CONV significantly reduced tumor volume ($p < 0.001$), tumors becoming undetectable by day 14 post-irradiation compared to controls (Figure 1).
- Tumor recurrence began around day 25 in all treated groups. In wild type C57BL/6 mice, CONV showed a trend towards better tumor control with smaller recurrent tumors and some complete responses but it caused severe skin toxicity, including inflammation and cracking, requiring euthanasia by day 50-60 (toxicity score: 5).

- FLASH RT was equally effective in long-term tumor control and markedly reduced skin toxicity, with only mild lesions observed in 3 of 10 mice (score: 2) (Figure 2 and 3).
- Notably, cGAS-/- mice exhibited mild toxicity with CONV RT and none in FLASH RT (score: 0) (Figure 2 & 3).

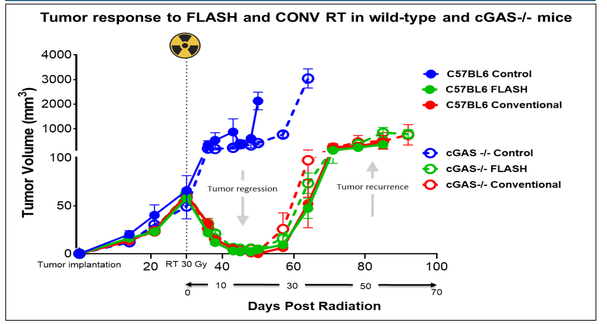


Figure 1. Tumor growth kinetics following RT in C57BL/6 and cGAS-/- mice: Both FLASH and CONV RT induced a marked reduction in tumor volume by day 14, followed by regrowth after day 25 post-radiation. In C57BL/6 mice (solid lines, filled circles) and cGAS-/- mice (dotted lines, open circles), tumor control was comparable between FLASH and CONV RT. Data are shown as mean ± SEM.

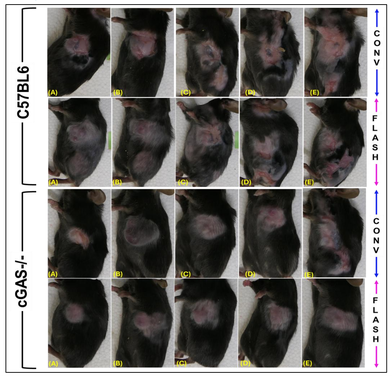


Figure 2. Pictures of tumor response and skin toxicity in C57BL/6 & cGAS-/- mice 50 days after receiving RT: In C57BL/6 mice, CONV RT produced marked skin damage & ulceration and erythema, whereas FLASH RT preserved skin integrity despite comparable tumor regression. In contrast, cGAS-/- mice exhibited minimal or no visible skin toxicity in both treatment groups, consistent with reduced inflammatory responses.

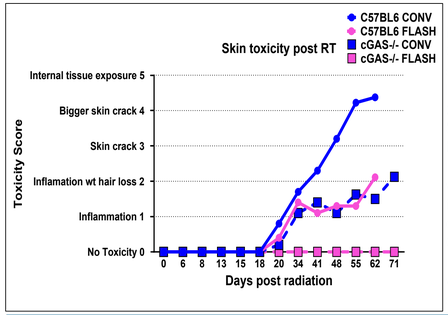


Figure 3: Skin toxicity Post-RT: C57BL/6 CONV showed significantly high toxicity, (euthanised at days 50–60). FLASH-treated C57BL/6 had lower toxicity, with euthanasia due to tumor burden. In cGAS-/- mice, CONV showed minimal toxicity, while FLASH showed no toxicity.

Discussion

Both FLASH and CONV RT effectively reduced tumor burden, but FLASH markedly minimized normal tissue toxicity, supporting the "FLASH effect." The absence of skin inflammation in cGAS-/- mice suggests that the cGAS–STING pathway contributes to radiation-induced inflammation and tissue injury. These findings indicate that ultra-high dose rate delivery may spare normal tissue by modulating cGAS-STING signaling, without compromising tumor control. Targeting the cGAS pathway may therefore enhance RT selectivity and improve clinical outcomes by reducing toxicity while maintaining antitumor efficacy.

Conclusions

- FLASH RT significantly reduced normal tissue toxicity compared with CONV RT. Although CONV RT showed stronger initial tumor control, its use was limited by severe skin toxicity.
- The absence of toxicity in cGAS-/- mice suggests that the cGAS pathway contribute to radiation-induced inflammation.
- These findings support FLASH RT as a promising, less toxic therapeutic strategy and highlight the potential of modulating cGAS pathway to improve RT outcomes in BC.

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