Evaluating FLASH and Conventional radiation response on brain-resident microglia

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Ultra-high dose-rate FLASH radiotherapy (FLASH-RT) has emerged as a promising approach in oncology, potentially enhancing the therapeutic index by minimizing normal tissue toxicity while maintaining anticancer efficacy compared to conventional radiotherapy (CONV-RT). However, the radiobiological effects on cranial radiotherapy are yet to be fully understood. This study investigates the *in vivo* effects of FLASH-RT and CONV-RT on brain-resident microglia, which play a crucial role in maintaining central nervous system (CNS) homeostasis and serve as key markers of neuroinflammation. To study microglial response towards irradiation, we employed intravital two photon imaging in mice expressing green fluorescent protein (GFP) under the fractalkine receptor promoter (CX3CR1GFP/+), following cranial window implantation. Two weeks post-implantation, the animals received a single cranial irradiation dose of 15 Gy using electron beams at either a FLASH dose rate of 257 ± 2 Gy/s or a CONV dose rate of 4 ± 0.02 Gy/s. *In vivo* two-photon imaging was conducted at multiple time points: pre-irradiation, 1–2 hours post-irradiation, and on days 7, 14, 21, and 28 post irradiation. Our results reveal that both FLASH-RT and CONV-RT induced notable changes in microglial dynamics and morphology, with CONV-RT exerting a more pronounced impact. These findings provide essential insights into the effects of radiotherapy on brain-resident microglia, contributing to our understanding of CNS responses to radiation and paving the way for improved radiotherapeutic approaches.