

● PERSPECTIVE

Targeting colony stimulating factor 1 receptor to prevent cognitive deficits induced by fractionated whole-brain irradiation

Whole-brain irradiation (WBI), usually delivered in 25–30 fractions to accumulate a total dose of 55–60 Gy, is commonly used for the treatment of primary tumors in the central nervous system (CNS) and brain metastases. With improved treatment techniques patients have prolonged overall survival, but are more likely to experience late adverse effects. In contrast to other symptoms that concur with treatment, cognitive impairments develop in 50–90% of long term survivors (> 6 months) after WBI and are often progressive and irreversible (Greene-Schloesser and Robbins, 2012). The mechanisms responsible for the progressive cognitive impairments are poorly understood, and there is no clinical treatment to prevent or reduce these adverse effects.

We have previously shown that a single dose of 10 Gy WBI induces accumulation of periphery monocytes in the CNS starting 7 days following WBI (Morganti et al., 2014). In line with these findings, previous studies have shown that WBI induces infiltration and activation of immune cells that express MHC II, CD11c, CD3 (Moravan et al., 2011). Although Han et al. (2016) did not observe monocyte-derived macrophages in the acutely irradiated adult brain, the incongruence could be due to the different radiation sources and the methods used for monocyte quantification. We recently demonstrated that after fractionated WBI (fWBI) treatment with a specific colony stimulating factor 1 receptor (CSF-1R) inhibitor reduces the number of resident and peripherally derived mononuclear phagocytes at the time of irradiation and prevents the development of memory deficits and dendritic spine loss at later time points in mice (Feng et al., 2016). Here we discuss our results and recent reports by others focusing on targeting CSF-1R to ameliorate radiation-induced cognitive deficits, and provide perspectives for future investigations.

Modulating WBI induced cognitive deficits: microglia or monocytes? The CSF-1 signaling is essential for the survival, proliferation and function of mononuclear phagocytes (Chitu et al., 2016). Its receptor, CSF-1R, is expressed on the surface of both microglia in the CNS and monocytes in the periphery. As a result, CSF-1R inhibition has profound impact on both populations. In mice treated with CSF-1R inhibitors, microglia can be stably diminished depending on the dose used. Treatment with PLX5622, a CSF-1R specific inhibitor, supplemented in the chow at 300 ppm, results in 30–50% reduction of microglia (Dagher et al., 2015; Feng et al., 2016) while treatment with higher dose of PLX5622 at 1,200 ppm, or PLX3397 (an analog of PLX5622) at 290 ppm results in nearly full elimination (> 95%) of microglia (Spangenberg et al., 2016). Both partial and full elimination of microglia prevent WBI induced dendritic spine loss and the development of cognitive deficits (Acharya et al., 2016; Feng et al., 2016). Notably dendritic spine loss is

not detected at two weeks post fWBI but is apparent at one month when also cognitive deficits are measured, and persists to several months after fWBI (Feng et al., 2016 and Feng, Rosi unpublished data). While it is known that radiation induces microglia activation and infiltration of peripherally derived monocytes (Belarbi et al., 2013; Morganti et al., 2015), the mechanisms by which WBI and fWBI induces dendritic spine loss are not known. We have previously reported that WBI induces elevated levels of the chemoattractant cytokine CCL2 in the brain which allows CNS accumulation of peripherally derived CCR2+ monocytes (Morganti et al., 2014). CCR2 is a chemokine receptor expressed in blood derived monocytes but not in brain resident microglia (Morganti et al., 2015) and activation of the CCR2-CCL2 signaling axis has been shown to recruit CCR2-expressing monocytes (“inflammatory monocytes”) into the injured tissue where they become activated macrophages, expressing multiple proinflammatory mediators (Prinz and Priller, 2010). CCR2 deficiency prevents the emigration of CCR2+ Ly6C^{high} monocytes from the bone marrow into the blood stream (Saederup et al., 2010) and prevents neuronal and cognitive dysfunctions induced by WBI (Belarbi et al., 2013). PLX5622 treatment both at high and low dose results in partial depletion of Ly6C^{high} “inflammatory” monocytes in the blood while the Ly6C^{low} patrolling monocyte population remains unchanged (Feng et al., 2016). Consequently, fWBI-induced Ly6C^{high} monocyte accumulation in the CNS is reduced by CSF-1R antagonism. In the periphery Ly6C^{high} cells enter injured or infected tissues and differentiate into macrophages or dendritic cells. It has been hypothesized that in neuroinflammatory models such as traumatic brain injury and experimental autoimmune encephalomyelitis (a model for multiple sclerosis), Ly6C^{high} monocytes initiate and maintain inflammatory responses upon entering the CNS while microglia are involved in tissue remodeling (Saederup et al., 2010). Activation of these myeloid cells might add to the neuronal damage caused directly by WBI. However, based on previous reports we cannot draw a definitive conclusion as to whether microglia or monocytes are the main contributors that are responsible for persistent neuroinflammation after WBI and fWBI. It is possible that both populations directly and/or indirectly are involved in modulating cognitive functions. Further studies selectively targeting either population can help to define their functions.

CSF-1R inhibition as potential therapy to prevent WBI induced cognitive deficits: While the exact mechanisms are unclear the fact that CSF-1R inhibition prevents WBI and fWBI-induced cognitive deficits remains extremely important for treatment strategy. However, further preclinical studies are necessary to assess the safety and efficacy of CSF-1R inhibitors. First, CSF-1R signaling plays different roles in young and old brains. Cortical neurons, immature neurons, neural progenitor cells during development and after chemical injury express CSF-1R (Chitu et al., 2016). Therefore, targeting CSF-1R under these conditions might directly affect neuronal functions and should be considered when planning CSF-1R therapy. Second, microglia express CSF-1R but not CCR2, while monocytes express both receptors that are involved in their survival and migration. Therefore, CSF-1R inhibition is likely to cause differential responses in contexts of CNS and peripheral inflam-

mation. Although there is increasing evidence showing that blocking CSF-1R drastically reduces microglia, it is unclear how CSF-1R inhibition can influence monocytes and contribute to periphery inflammatory response. During PLX5622 treatment, we observed about 30% reduction of Ly6C^{high} monocytes but not Ly6C^{low} monocytes. However, reports by others show that CSF-1R antibody depleted the nonclassical CD14⁺CD16⁺ monocytes but not the classical CD14⁺CD16⁻ monocytes in human (Ries et al., 2014). These differences could be caused by a number of reasons such as usage of small inhibitor compound vs. antibody, the specificity of the inhibitor/antibody and different responses to CSF-1R inhibition between rodents and human. The possible crosstalk between CSF-1/CSF-1R and CCL2/CCR2 signal pathways is currently unknown. Third, patients usually experience cognitive decline several months or years after receiving WBI. However, in published animal studies, CSF-1R inhibition lasted from 7 days to 12 weeks. It is unclear whether transient CSF-1R inhibition during irradiation is enough to prevent long-term cognitive deficits after WBI in humans or mice. In addition, macrophages may play different roles in different microenvironments. Infiltrating monocytes/macrophages may switch their functions from promoting clearance of debris and tissue damage into wound healing and tissue repair over time. Further studies are needed to optimize the treatment window and understand potential long-term side effects of CSF-1R inhibition. Finally, the currently published data look at WBI models in tumor-free conditions, focusing on radiation effects on normal brains. However, brain tumor patients represent the largest population who receive whole brain irradiation, and they often experience surgery followed by chemotherapy and fractionated radiotherapy. CSF-1R inhibitors target tumor associated macrophages, the most abundant immune cells in brain tumors. Therefore, it is possible that CSF-1R inhibitor may interact with brain tumors by interfering with both on-going therapies and tumor microenvironment. It has been reported that CSF-1R inhibitor alters the polarization of tumor associated macrophages and blocks glioma progression (Pyonteck et al., 2013), but more than half of tumors recur after prolonged treatment (Quail et al., 2016). Although these results suggest overall benefits in survival after CSF-1R inhibitor treatment, the cognitive outcomes are unknown. Therefore, preclinical studies in brain tumor models that recapitulate clinical conditions are necessary to examine these issues.

This work was supported by National Institutes of Health (NIH), grants: R01CA213441; R01CA133216 and the Pediatric Brain Tumor Foundation Institute Grant.

Xi Feng, Susanna Rosi*

Brain and Spinal Injury Center, Weill Institute for Neuroscience, Kavli Institute of Fundamental Neuroscience, Department of Physical Therapy and Rehabilitation Science, Department of Neurological Surgery, University of California, San Francisco, CA, USA

*Correspondence to: Susanna Rosi, Ph.D., susanna.rosi@ucsf.edu.

Accepted: 2017-02-28

orcid: 0000-0002-9269-3638 (Susanna Rosi)

0000-0002-6920-1519 (Xi Feng)

doi: 10.4103/1673-5374.202940

How to cite this article: Feng X, Rosi S (2017) Targeting colony stimulating factor 1 receptor to prevent cognitive deficits induced by fractionated whole-brain irradiation. *Neural Regen Res* 12(3):399-400.

Open access statement: This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

References

- Acharya MM, Green KN, Allen BD, Najafi AR, Syage A, Minasyan H, Le MT, Kawashita T, Giedzinski E, Parihar VK, West BL, Baulch JE, Limoli CL (2016) Elimination of microglia improves cognitive function following cranial irradiation. *Sci Rep* 6:31545.
- Belarbi K, Jopson T, Arellano C, Fike JR, Rosi S (2013) CCR2 deficiency prevents neuronal dysfunction and cognitive impairments induced by cranial irradiation. *Cancer Res* 73:1201-1210.
- Chitu V, Gokhan Ş, Nandi S, Mehler MF, Stanley ER (2016) Emerging roles for CSF-1 receptor and its ligands in the nervous system. *Trends Neurosci* 39:378-393.
- Dagher NN, Najafi AR, Kayala KM, Elmore MR, White TE, Medeiros R, West BL, Green KN (2015) Colony-stimulating factor 1 receptor inhibition prevents microglial plaque association and improves cognition in 3xTg-AD mice. *J Neuroinflammation* 12:139.
- Feng X, Jopson TD, Paladini MS, Liu S, West BL, Gupta N, Rosi S (2016) Colony-stimulating factor 1 receptor blockade prevents fractionated whole-brain irradiation-induced memory deficits. *J Neuroinflammation* 13:215.
- Greene-Schloesser D, Robbins ME (2012) Radiation-induced cognitive impairment--from bench to bedside. *Neuro-oncology* 14 Suppl 4:iv37-44.
- Han W, Umekawa T, Zhou K, Zhang XM, Ohshima M, Dominguez CA, Harris RA, Zhu C, Blomgren K (2016) Cranial irradiation induces transient microglia accumulation, followed by long-lasting inflammation and loss of microglia. *Oncotarget* 7:82305-82323.
- Moravan MJ, Olschowka JA, Williams JP, O'Banion MK (2011) Cranial irradiation leads to acute and persistent neuroinflammation with delayed increases in T-cell infiltration and CD11c expression in C57BL/6 mouse brain. *Radiat Res* 176:459-473.
- Morganti JM, Jopson TD, Liu S, Riparip LK, Guandique CK, Gupta N, Ferguson AR, Rosi S (2015) CCR2 antagonism alters brain macrophage polarization and ameliorates cognitive dysfunction induced by traumatic brain injury. *J Neurosci* 35:748-760.
- Prinz M, Priller J (2010) Tickets to the brain: role of CCR2 and CX3CR1 in myeloid cell entry in the CNS. *J Neuroimmunol* 224:80-84.
- Pyonteck SM, Akkari L, Schuhmacher AJ, Bowman RL, Sevenich L, Quail DF, Olson OC, Quick ML, Huse JT, Teijeiro V, Setty M, Leslie CS, Oei Y, Pedraza A, Zhang J, Brennan CW, Sutton JC, Holland EC, Daniel D, Joyce JA (2013) CSF-1R inhibition alters macrophage polarization and blocks glioma progression. *Nat Med* 19:1264-1272.
- Quail DF, Bowman RL, Akkari L, Quick ML, Schuhmacher AJ, Huse JT, Holland EC, Sutton JC, Joyce JA (2016) The tumor microenvironment underlies acquired resistance to CSF-1R inhibition in gliomas. *Science* 352:aad3018.
- Ries CH, Cannarile MA, Hoves S, Benz J, Wartha K, Runza V, Rey-Giraud F, Pradel LP, Feuerhake F, Klamann I, Jones T, Jucknischke U, Scheiblich S, Kaluza K, Gorr IH, Walz A, Abiraj K, Cassier PA, Sica A, Gomez-Roca C, et al. (2014) Targeting tumor-associated macrophages with anti-CSF-1R antibody reveals a strategy for cancer therapy. *Cancer Cell* 25:846-859.
- Saederup N, Cardona AE, Croft K, Mizutani M, Cotleur AC, Tsou C-L, Ransohoff RM, Charo IF (2010) Selective chemokine receptor usage by central nervous system myeloid cells in CCR2-red fluorescent protein knock-in mice. *PLoS One* 5:e13693.
- Spangenberg EE, Lee RJ, Najafi AR, Rice RA, Elmore MR, Blurton-Jones M, West BL, Green KN (2016) Eliminating microglia in Alzheimer's mice prevents neuronal loss without modulating amyloid-beta pathology. *Brain* 139:1265-1281.