Pivotal Role of Cranial Irradiation-Induced Peripheral, Intrinsic, and Brain-Engrafting Macrophages in Malignant Glioma

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ABSTRACT: Malignant (high-grade) gliomas are aggressive intrinsic brain tumors that diffusely infiltrate the brain parenchyma. They comprise of World Health Organization (WHO) grade III and IV gliomas. Ionizing radiation or irradiation (IR) is frequently utilized in the treatment of both primary as well as metastatic brain tumors. On the contrary, macrophages (MΦ) are the most copious infiltrating immune cells of all the different cell types colonizing glioma. MΦ at tumor milieu are referred to as tumor-associated macrophages (TAMΦ). In malignant gliomas milieu, TAMΦ are also polarized into two distinct phenotypes such as M1 TAMΦ or M2 TAMΦ, which are capable of inhibiting or promoting tumor growth, respectively. Cranial-IR such as x- and γ-IR are sufficient to induce the migration of peripherally derived MΦ into the brain parenchyma. The IR facilitate a more immunosuppressive milieu via the stimulation of efferocytosis in TAMΦ, and an upsurge of tumor cell engulfment by TAMΦ exhibited detrimental effect of the anti-tumoral immune response in glioma. The MΦ inside the tumor mass are associated with multiple phenomena that include IR resistance and enrichment of the M2 MΦ after IR is able to facilitate glioblastoma multiforme (GBM) recurrence. Reviews on the role of cranial IR-induced peripheral and brain-engrafting macrophages (BeMΦ) in glioma are lacking. Specifically, most studies on peripheral, intrinsic as well as beMΦ on IR focus on WHO grade III and IV. Thus, this review precisely focuses primary on WHO grade III as well as IV gliomas.

KEYWORDS: Irradiation, glioma, malignant, macrophages, radiosensitization, radioresistance

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Introduction

Malignant (high-grade) gliomas are aggressive intrinsic brain tumors that diffusely infiltrate the brain parenchyma.[1-4] They are rapidly progressive comprising of World Health Organization (WHO) grade III that includes anaplastic astrocytoma, anaplastic oligodendroglioma, mixed anaplastic oligoastrocytoma, and WHO grade IV, also referred to as glioblastoma multiforme (GBM).[2,3,5] The incidence of high grade is about 5 out of 100,000 and they are made up of 35% to 45% of all primary brain tumors.[1,6] Notably, anaplastic astrocytomas constitute about 10% to 15%, while anaplastic oligodendrogliomas as well as anaplastic oligoastrocytomas constitute about 10% of all malignant gliomas.[3,6]

The GBM is mostly common in adults and it is still the most challenging brain tumor clinically.[7-9] Epidemiologically, GBM constitutes about 14.5% of all central nervous system (CNS) tumors and 48.6% of malignant CNS tumors with a median overall survival as low as 15 months.[7,10] Pathologically, it is an extremely heterogeneous tumor with distinct coexisting cell categories such as tumor cells, fibroblasts, endothelial cells, as well as diverse immune cells.[11] Standard treatments modalities comprise of utmost safe surgery followed by external radiotherapy as well as simultaneous chemotherapy.[12,13] Notably, current mechanical and treatment scheme targets the tumor cells and neglects other cellular components such as macrophages (MΦ) recruited to the GBM or tumor milieu.

Ionizing radiation or irradiation (IR) is frequently utilized in the treatment of both primary as well as metastatic brain tumors.[14,15] Notably, whole-brain radiotherapy (WBRT), delivered in multiple fractions, is routinely utilized for the treatment of patients with both primary as well as metastatic brain tumors.[14,15] On the contrary, MΦ are the most copious infiltrating immune cells of all the different cell types colonizing GBM. Notably, MΦ at tumor milieu are referred to as tumor-associated macrophages (TAMΦ).[16] Interestingly, circulating monocytes are capable of migrating to the tumor milieu and once in tumor milieu, they segregate into MΦ (M0 MΦ) via the stimulation cytokines.[17] Intriguingly, M0 MΦ show a high level of plasticity and are polarizable into two distinct functional phenotypes such as M1 MΦ and M2 MΦ once differentiated.[18]

In GBM milieu, TAMΦ are also polarized into two distinct phenotypes such as M1 TAMΦ or M2 TAMΦ, which are

capable of inhibiting or promoting tumor growth, respectively.[19-21] Notably, M1 TAMΦ are proinflammatory and they generate high quantities of inducible nitric oxide synthase (iNOS) whereas M2 TAMΦs are anti-inflammatory, pro-angiogenic, accelerates metastasis, and generate high quantities of Arg I.[21] Remarkably, primary TAMΦ function as M1, but are progressively changed to M2 as the tumor advances.[22] Notably, M1 and M2 TAMΦ dominate in distinctive areas of the tumor milieu, with M2 TAMΦ migrating and accumulating in avascular and hypoxia regions.[19,23] Thus, M1/M2 TAMΦ ratio may vary in different parts of the tumor.

In GBM microenvironment, TAMΦs are very crucial during the recurrence of GBM and their presence signifies tumor aggressiveness as well as of the overall survival of those patients with GBM.[24] Interestingly, WBRT causes detrimental effects to the CNS milieu leading to the accumulation of peripherally derived MΦ.[14,25] However, reviews on the pivotal role of cranial IR-induced peripheral and brain-engrafting macrophages (BeMΦ) in glioma are lacking. Specifically, most studies on peripheral, intrinsic, as well as beMΦ on IR focus on WHO grade III and IV. Thus, this review precisely focuses primarily on WHO grade III as well as IV gliomas.

Literature Search Method and Scope of Review

The "Boolean logic" was used to search for article on the subject matter in PubMed and PubMed central as well as google scholar with search terms such as cranial IR and/or peripheral, intrinsic, beMΦ and/or glioma. Thus, this review discuses *in vivo* as well as *in vitro* studies that describe the association between cranial IR and peripheral, intrinsic, beMΦ in glioma at the bench level. Studies on microglia were excluded because this review focuses primarily on the effect of cranial IR on peripheral, intrinsic, as well as beMΦ in glioma. However, to clearly differentiate between microglia and other MΦ, articles that differentiate between the two were discussed. Also, articles that discuss MΦ and IR-induced abscopal and bystander effects were discussed.

Notably, articles that define or describe specific factors but are not related to cranial IR and peripheral, intrinsic, beMΦ in glioma were acknowledged and cited accordingly. Importantly, articles that describe the association between cranial IR and peripheral, intrinsic, beMΦ in glioma are discussed in subheadings such as, (1) residual MΦ, microglia, and BeMΦ, (2) MΦ at Tumor and IR Tumor Milieu, (3) IR and MΦ subtypes, (4) IR-induced hypoxia and MΦ signaling pathways, (5) MΦ induce radiosensitization and radioresistance, and (6) MΦ and IR-induced abscopal and bystander effects in accordance to how research is conducted at bench level. Conclusions and perspectives were drawn from the subheading above.

Residual MΦ, Microglia, and BeMΦ

Residual brain MΦ are characterized into perivascular, meningeal, circumventricular organs, and choroid plexus MΦ based their anatomical locations.[19,26] Also, all residual brain MΦ are located in the perivascular or Virchow-Robin spaces, subdural meninges, whereas choroid plexus originated from short-lived blood monocytes after birth, which are rapidly replaced by bone marrow derived cells.[19,26,27] Furthermore, perivascular and meningeal MΦ are produced from embryonic yolk sac precursors, whereas choroid plexus MΦ have dual embryonic and adult haematopoietic origins.[26] Moreover, residual brain MΦ are often restricted at the boundary between the parenchyma and the circulation.[19]

Microglia are the original phagocytes of the brain as well as spinal cord and they are heterogeneously situated in almost all non-overlapping sections of the brain and the spinal cord.[28,29] Notably, they are responsible for the detection and engulfing of extracellular components like cell debris, apoptotic cells, tumors cells and microbes.[19,28] Also, microglia usually communicate with neuronal circuits in developing and in the adult brain.[29,30] Similarly, microglia stimulate neuronal apoptosis, eliminate less functional synaptic connections like synaptic trimming and induce neuronal activity.[31,32] It was observed that in GBMs, TAMΦ were not brain-resident microglia, but mainly monocyte-derived MΦ from peripheral blood in GBM milieu.[33]

It is worth noting that obvious differences in functions, abundance, as well as spatial distribution have been identified between beMΦ and microglia.[34] In addition, beMΦ such as monocyte-derived MΦ and TAMΦ as well as microglia have been investigated separately and their distinct functions in GBM pathogenesis observed using single-cell omics and fate-mapping systems.[35] Furthermore, human GBM was mainly infiltrated by monocyte-derived MΦ rather than microglia during a single-cell immunohistochemical analysis.[36] Moreover, beMΦ were spatially distributed in the center of the tumor while microglia were spatially distributed at the invasive margin.[34]

Also, beMΦ were capable of expressing more pro-inflammatory factors than microglia during single-cell and bulk gene expression data analysis.[37] Notably, beMΦ signatures such as gene expression and epigenetic marks were precipitously lost upon *ex vivo* culture, as were best recognized in microglia.[38] Similarly, beMΦ expressed genes such as C-C motif chemokine receptor 2 (CCR2), interferon alpha and beta receptor subunit 1(IFNAR1), membrane Spanning 4-domains A7 (MSA4A7) and apolipoprotein E (Apo-E) that were absent from host microglia, and exhibited MΦ scavenger receptor 1 (MSR1) as well as anexelekto (AXL) mRNAs that correlated with the absence of spalt-like transcription factor 1 (SALL1).[39] Moreover, transcriptomes analysis of beMΦ revealed similarity with perivascular MΦ and deficiency of a regulatory pathway stimulated by interleukin (IL)-10, as compared to host microglia.[40]

Colony-stimulating factor 1 receptor (CSF1R) is a receptor tyrosine kinase responsible for development as well as functions of myeloid cells such as monocytes and MΦ.[41,42] Comparative transcriptome analysis revealed that beMΦ in mice tentatively depleted microglia due to a CSF1R deficiency maintaining a transcriptional identity different from host cells.[43] Also, selective depletion of resident microglia without blood–brain barrier (BBB) disruption triggered a permissive

environment for the recruitment, infiltration, as well as the engraftment of peripheral MΦ.[14,44] Thus, peripherally derived MΦ were capable of spatially replacing microglia as well as developed ramifications analogous to microglia.[45]

It is worth noting that beMΦ sustain their distinctive transcriptional as well as functional uniqueness in three different beMΦ engraftment models.[43] Also, persistent loss of microglia as well as their failure to repopulate the niche was adequate to stimulate beMΦ engraftment into the brain and spinal cord in the absence of IR.[43] Furthermore, beMΦ were able to replace microglia only when microglia are compromised in their capability to repopulate the niche, without the need for IR, inflammation, or BBB disruption.[43] Moreover, spatial distribution of microglia and MΦ stimulated differential GBM cells and their viability to phagocytes after IR exposure.[43]

MΦ at Tumor and IR Tumor Milieu

Gliomas are histologically classified into Grades I to IV according to the WHO criteria.[46,47] Also, based on histological classification above, Grade III comprises astrocytoma or anaplastic astrocytoma, whereas GBM forms Grade IV gliomas.[48] Furthermore, GBM are also further classified into isocitrate dehydrogenase (IDH)-wild type, IDH-mutant, not-otherwise-specified and not-elsewhere-classified.[49,50] Similarly, the IDH-wild type constitutes about 90% of cases and it *de novo* starts at about 60 years of age, whereas the IDH-mutant constitutes about 10% of cases and is often a secondary GBM.[49,50]

These secondary GBM usually develop in younger patients with gliomas of higher differentiation such as WHO Grades I to III.[50,51] It is worth noting that IDH-mutant carries an expressively better prognosis than wild type IDH.[51] Also, in not-otherwise-specified type, the IDH mutation status is often not determined because of lack of histological or molecular material for testing, whereas the not-elsewhere-classified is the fourth type that was identified in recent years.[49,50] Thus, most studies on peripheral, intrinsic, as well as beMΦ on IR in glioma focus on grades III and IV.

Notably, specific molecular subtypes of gliomas are capable of influencing the abundance as well as functional characteristics of MΦ.[35] Also, grade IV or GBM tend to contain a higher abundance of TAMΦ than other subtypes of glioma.[52] Similarly, a single-cell RNA sequencing (scRNA-seq) analysis has shown that recurrent GBM has more infiltrating TAMΦ than primary GBM.[53] Moreover, higher MΦ enrichment was detected in the mesenchymal subtype of GBM than in the proneural as well as classical subtypes.[54] Furthermore, the difference above was due to the NF1 mutation typically seen in mesenchymal GBM as NF1 was capable of modulating myeloid cell chemotaxis.[54]

In addition, stimulated MΦ were capable of triggering the secretion of proteolytic enzymes as well as inflammatory cytokines.[55] Also, MΦ, which are inflammatory cells in GBM, facilitated tumor development as well as symbolized a negative prognostic factor.[16,56] It is worth noting that mutations in *IDH* genes were capable of influencing MΦ infiltration in GBM. Precisely, IDH wild type had a higher level of infiltrating MΦ compared to IDH-mutant GBM during scRNA-seq analysis.[35] Similarly, better prognosis was observed in IDH-mutant GBM patients compared to IDH-wild type patients GBM.[35]

Notably, TAMΦ are recruited during the early stage of GBM tumorigenesis and they are mostly located in the perivascular regions.[57] In GBM milieu, TAMΦ expressed higher concentration of major histocompatibility complex (MHC)-II but could not interact with T cells localized in the tumor periphery.[35,58] Furthermore, negative correlation between TAMΦ infiltration and survival was observed in adult patients with GBM.[59] However, contradictory study revealed positive correlation between $CD68^+$ $CD163^+$ $CD206^+$TAMΦ infiltration and the overall survival of patients with IDH1R132H-wild type GBM.[60]

Interestingly, immunostaining of MΦ and small vessels in resected glioma specimens revealed an augmented numbers of infiltrating MΦ and small vessel density in GBM compared to astrocytomas or anaplastic astrocytomas.[61] Also, MΦ infiltration correlated with vascular density in human gliomas and heme oxygenase-1 (HO-1), a rate-limiting enzyme in heme catabolism, was also linked to the stimulation of MΦ.[61] Moreover, secretion of mRNA encoding HO-1 correlated with MΦ infiltration as well as vascular density in human glioma.[61] In addition, infiltrating MΦ were positively stained with anti-HO-1 antibody via immunohistochemical analysis, as well as *in situ* hybridization for HO-1 revealed that HO-1 was secreted in infiltrating MΦ in gliomas.[61] Thus, *HO-1* gene is a promising marker for MΦ infiltration as well as neovascularization in human gliomas.

Cranial-IR such as X- and γ-IR are sufficient to induce the migration of peripherally derived MΦ into the brain parenchyma.[14,62] Also, an acute response to IR exposure was responsible for the induced peripheral MΦ immigration into the brain in seven-day time.[14] It is worth noting that IR was capable of influencing the tropism of MΦ in the tumor by augmenting the generation of chemokines at the origin of MΦ migration.[14,62] It is observed that beMΦ underwent clonal proliferation and thereby likely progressively outcompeted IR.[14,62] Also, tissue MΦ such as Kupffer cells and alveolar MΦ were capable of replacing by bone marrow-derived cells in IR chimeras and other small animal models deficient of resident MΦ.[63] Ionized calcium binding adaptor molecule 1 (Iba1) is a MΦ-specific calcium-binding protein.[64]

It is worth noting that beMΦ enter the brain 14 days after the completion of brain IR, or 4 days after the CSF-1Ri withdrawal.[25] Moreover, the ratio of beMΦ was high at 14 days after the completion of brain IR but with no restoration of the total number of Iba1 positive cells.[25] Furthermore, stromal cell-derived factor 1 (SDF-1) is a member of the CXC group of chemokines (CXCL12) and it is an endogenous ligand for the

chemokine receptor CXCR4. In addition, IR facilitated the recruitment of MΦ in GBM practically 20 days post-IR by augmenting the SDF-1 production.[65,66] Moreover, the early response after IR in high-grade glioma was depicted with astrocytic gliosis, vascular proliferation, and infiltration of MΦ.[67]

IR and MΦ Subtypes

It is worth noting that MΦ are also capable of polarizing into two distinct functional phenotypes such as M1 MΦ and M2 MΦ once differentiated following IR exposure.[62] MΦ described herein are beMΦ. Distinctly, x-IR exposure triggered a local reoxygenation resulting in modulation of MΦ phenotype.[68,69] Also, while IR was capable of augmenting M1 MΦ markers in earlier study,[70] a later study failed to detect any alteration in cytokine production.[71] Similarly, augmentation in M2 MΦ markers was detected in GBM in *in vivo* models of MΦ following exposure to IR.[20] Furthermore, x-IR exposure triggered a loss of MΦ present in GBM and the percentage of M2 MΦ such as $CD206^+$ cells relative to total MΦ such as $CD68^+$ cells was increased after IR.[62]

Interestingly, $CD68^+$ cells were detected outside the tumor core signifying that $CD68^+$ cells were recruited within the GBM.[66,72] Also, an upsurge in M2 MΦ population selectively triggered cell death of M0 as well as M1 MΦ and compared to M0 as well as M1 MΦ, M2 MΦ were less sensitive to IR during *in vitro* experiments.[62] In addition, M0 and M1 MΦ were capable of repairing DNA DSBs although their proportion were decreased post-IR.[62] Thus, M0 and M1 MΦ misrepair DNA DSBs resulted in certain genomic instabilities. Moreover, x-IR was also capable of augmenting M2 MΦ percentage in a recurrent GBM model.[62] It is worth noting that an upsurge in MΦ migration, which was parallel to an upsurge in M2 MΦ quantity, was detected after 22 days post-IR at which time MΦ were recruited in GBM.[20,66]

Similarly, an upsurge in the quantity of M2 MΦ, which was the phenotype relatively resistant to IR, was detected before MΦ recruitment. Interestingly, M0 and M1 MΦ were more sensitive to IR than M2 MΦ.[62] Also, augmented secretion of p-extracellular signal-regulated kinase (p-ERK) and p-protein kinase B (p-AKT) were detected in M2 MΦ relative to M0 and M1 MΦ implying that M2 MΦ are more radioresistant.[62] Notably, p-ERK/pan-ERK as well as p-AKT/pan-AKT are recognized as two major players in radioresistance.[62] Also, M0 and M1 MΦ are usually located in oxygenated areas of GBM following IR.[68] Thus, a significant reduction in the number of M0 and M1 MΦ was observed in 20% O_2 *in vitro* GBM model following IR.

In addition, M0 MΦ quantity remained stable after IR whereas M1 MΦ were still reduced in 0.2% O_2, which is considered as severe hypoxia.[62] Moreover, decrease in DNA DSBs was observed in M0 MΦ at 0.2% O_2. This signifies that at low O_2 pressure, M0 MΦ were already programed toward an M2 MΦ.[68] Similarly, M1 MΦ produced significant concentrations of NO, which decreased hypoxia.[68] However, NO generated M1 MΦ was still superior to the quantity generated in M0 and M2 MΦ.[62] Also, M2 MΦ were capable of repairing DNA DSBs and were more radioresistant to x-IR.[62] Moreover, cell death was inhibited by M2 MΦ in hypoxic conditions thus allowing M2 MΦ to be formed at detriment of M0 and M1 MΦ.[68] Furthermore, M2 MΦ were associated to glioma stem cells (GSCs) in hypoxic areas and facilitated tumor development.[33]

IR Induce Hypoxia and MΦ Signaling Pathways

In GBM milieu, the most common non-neoplastic cells are TAMΦ, which are made up of peripheral MΦ or beMΦ, brain-intrinsic MΦ, and microglia.[73,74] Notably, TAMΦ are a critical component of the local tumor milieu, and they influence tumor immune evasion, suppress T cell activity, as well as control initiation, progression and metastasis.[75] Also, TAMΦ comprise about 30% of infiltrating cells and their infiltration is authentically associated with the outcome of GBM patients.[76] Furthermore, GBM milieu is influenced by immune-related signaling pathways such as innate immune cascade, inflammatory cascade, and complement cascade activation, via TAMΦ.[73] Principally, interferon-gamma (IFN-γ) and T-cell proliferation are most influenced via TAMΦ following IR in GBM (see Figure 1).[73]

MΦ are more resistant to IR than other cells and they react by augmenting the generation of reactive oxygen species (ROS) and NOS.[77] MΦ-induced ROS and NOS triggers the secretion of damage-associated molecular patterns (DAMP) from damaged cells, induce inflammatory transcription factors as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) as well as RelB resulting in the secretion of inflammatory cytokines and chemokines in GBM (see Figure 1).[67,77] This cascade leads to angiogenesis, edema, as well as tissue damage, but will also recruit more inflammatory cells by chemotaxis and thus alter the immune-milieu (see Figure 1).[67,77] Also, hypoxia was capable of decreasing ROS accumulation and this correlated with a reduction in M0 MΦ death following IR (see Figure 1).[62]

It is worth noting that hypoxic tumor milieu is mostly infiltrated by TAMΦ, which constitute the largest population of infiltrating inflammatory cells.[65] Also, the relationship between TAMΦ and hypoxia is believed to be bidirectional (see Figure 1).[20] Interestingly, IR-induced augmentation in TAMΦ in tumor core was essentially associated with the development of IR-induced chronic hypoxia.[20] Furthermore, IR-induced augmentation in TAMΦ at the tumor-invading front was triggered by mechanism other than chronic hypoxia, because this region exhibited higher microvascular density.[65]

In addition, hypoxia-induced factor-1-alpha (HIF-1α) stabilization triggered the secretion of angiogenic factor such as vascular endothelial growth factor (VEGF) and chemotactic

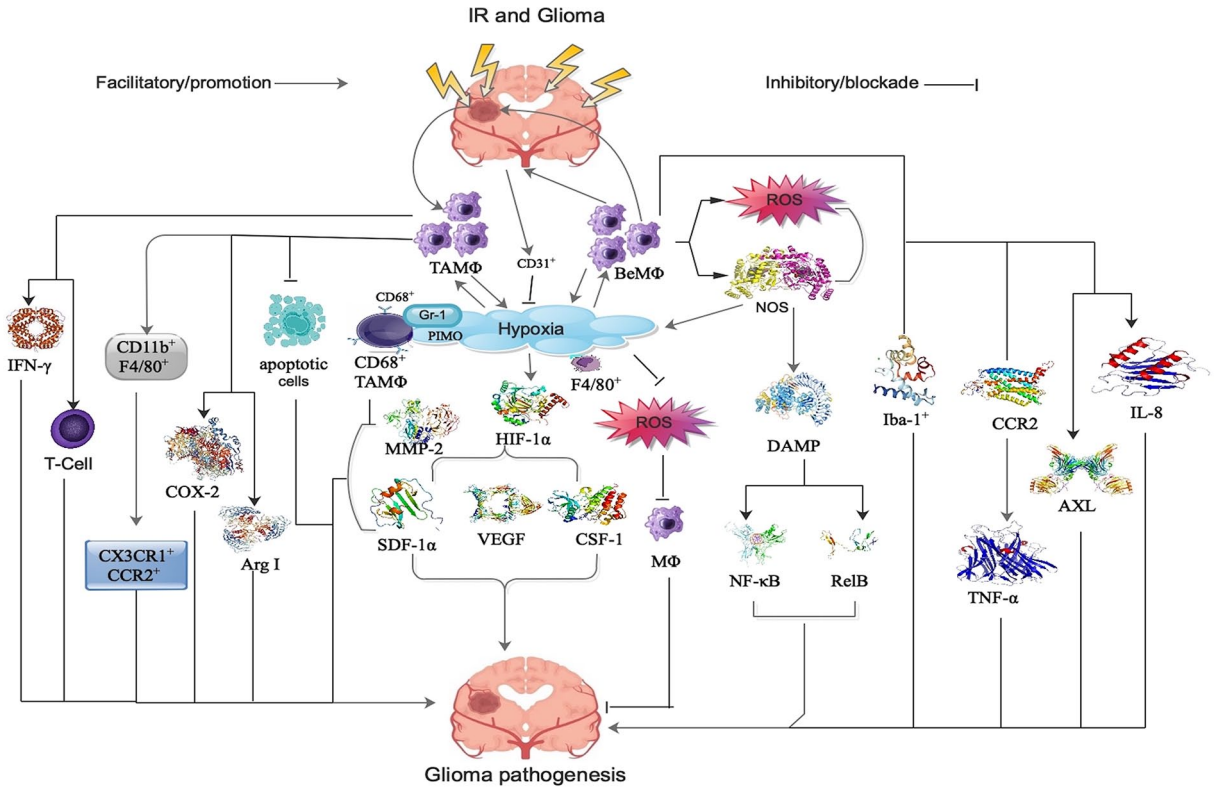

Figure 1. Shows the signaling modalities of cranial IR-induced peripheral MΦ, BeMΦ, as well as TAMΦ in the pathogenesis of glioma. Refer to the text for detailed explanations. AXL indicates anexelekto; BeMΦ, brain-engrafting macrophages; COX-2, cyclooxygenase-2; CSF, colony-stimulating factor; DAMP, damage-associated molecular pattern; HIF, hypoxia-induced factor; Iba, ionized calcium-binding adaptor; IFN, interferon-gamma; IL, interleukin; IR, ionizing radiation or irradiation or radiotherapy MMP, matrix metalloproteinase; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PIMO, pimonidazole; ROS, reactive oxygen species; SDF, stromal cell-derived factor; TAMΦ, tumor-associated macrophages; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

factors such as SDF-1α and CSF-1 by hypoxic tumor cells following IR (see Figure 1).[20,78] Notably, these factors also recruited peripheral MΦ to the hypoxic tumor milieu to restore blood delivery as well as nurture the hypoxic cells following IR.[20] Also, it was observed that the association between TAMΦ and hypoxia was not only tumor type dependent, but also stroma dependent.[20]

Interestingly, a majority of the TAMΦ in the primary tumor core were $CD68^{2+}$ as well as $F4/80^{2+}$.65 Also, TAMΦ in the invading tumor front were both $CD68^{2+}$ and $F4/80^{2+}$ as well as $F4/80^{+}$ and $CD68^{-}$ TAMΦ following IR (see Figure 1).[79] Furthermore, the hypoxic regions in IR tumors or tumors growing in pre-IR tissues had more $CD68^{+}$TAMΦ accumulation compared to control tumors in experiments involving ALTS1C1 astrocytoma and murine GL262 tumors models.[20] Notably, pimonidazole (PIMO) is a hypoxia marker for the detection of glioma aggressiveness and metastasis.

It is worth noting that $CD68^{+}$, but not $F4/80^{+}$ induced TAMΦ selectively amass in $PIMO^{+}$ hypoxia regions in intracranial ALTS1C1 astrocytoma following IR (see Figure 1).[20] Therefore, the association of $CD68^{+}$TAMΦ with hypoxia is tumor dependent. Contrarily, in murine GL261 brain glioma models, $CD68^{+}$TAMΦ did not have preference for $PIMO^{+}$ hypoxic regions.[20] Also, most $PIMO^{+}$ hypoxic regions in control tumors composed of $CD31^{+}$ vessels, signifying that abnormal vessel perfusion triggered hypoxia and this led to transient hypoxia.[20] Contrarily, most IR-induced hypoxic regions did not contain $CD31^{+}$ vessels, signifying that the hypoxia was triggered by insufficient blood vessels and this resulted in avascular chronic hypoxia (see Figure 1).[20]

The IR-induced hypoxic regions typically develop central necrosis resulting in the accumulation of positive gamma response 1 ($Gr\text{-}1^{+}$) neutrophils.[20] The Gr-1 is a marker for granulocytes. Also, pre- and post-IR alters tumor milieu in such a way that TAMΦ aggregate in hypoxic regions and $Gr\text{-}1^{+}$ neutrophils (see Figure 1).[20] Thus, $CD68^{+}$TAMΦ, and $F4/80^{+}$TAMΦ resegregate into different tumor milieu. It is worth noting that IR-induced hypoxic milieu may have specific factors that cause $CD68^{+}$TAMΦ aggregation because avascular chronic hypoxia was observed in larger control tumors, but no $CD68^{+}$TAMΦ aggregation.[20]

Interestingly, higher $Iba\text{-}1^{+}$ cells were detected at the invading tumor front compared with $CD68^{+}$, signifying that higher levels of mature MΦ were involved in the invasion of tumor front (see Figure 1).[20] Also, IR recruited the infiltration of more $CD68^{+}$ MΦ from peripheral blood and these IR-recruited

$CD68^+$ MΦ performed the role of M2 MΦ leading to the facilitation of tumor invasion (see Figure 1).[20] Moreover, ALTS1C1 and GL261 tumors were characterized with different $CD68^+$ TAMΦ–hypoxia association patterns, which were related to three monocytes-associated factors such as SDF-1α, VEGF, as well as matrix metalloproteinase-2 (MMP-2; see Figure 1).[20]

The IR was capable of inducing SDF-1α production, which facilitated the homing of hematopoietic progenitor cells toward gliomas as well as augmented vessel formation.[20,79] Moreover, SDF-1α in the conditioned medium generated by ALTS1C1 tumor not only augmented MΦ migration toward hypoxia, but also lengthen their survival in hypoxic milieu.[79] In addition, SDF-1α generation by tumor cells was capable of triggering the accumulation of TAMΦ in IR-induced hypoxic regions as it silenced ALTS1C1 tumor development in intramuscular or intracranial pre-IR sites.[20]

Inhibition of TAMΦ amassment in hypoxia and tumor development delay was further augmented in SDF-1α-suppressed tumors.[20] Thus, SDF-1α facilitated tumor development in an IR milieu and the association of TAMΦ with hypoxia augmented tumor development rate. Moreover, inhibition of SDF-1α secretion in ALTS1C1 tumors by siRNA triggered a reduction microvascular density, TAMΦ density, as well as tumor invasiveness following IR.[80] Therefore, IR-induced SDF-1α secretion was responsible for the IR-induced augmentation in microvascular density, infiltration of MΦ, and vessel vascularization, which subsequently triggered IR-induced tumor invasiveness.[20,65]

Hypoxia was capable of inducing iNOS secretion, which triggered TAMΦ migration in tumors (see Figure 1).[22] Also, TAMΦ isolated from IR tumors secreted higher levels of iNOS, arginase 1 (Arg I), as well as cyclooxygenase-2 (COX-2; see Figure 1) compared to un-IR tumors.[81] Interestingly, these factors were more effective in facilitating tumor growth.[81] Also, gene expression levels of CD11b, a marker for myeloid cells of the MΦ lineage, was significantly decreased in the hippocampus of IR mice 7 days post-IR.[14] Contrarily, MΦ markers such as $CD11b^+F4/80^+$ that co-secretes green fluorescent protein (GFP) as well as red fluorescent protein (RFP) such as $CX3CR1^+CCR2^+$ significantly increased 7 days following IR in tumors (see Figure 1).[14]

It is worth noting that blood-born monocytes and MΦ predominantly expressed CCR2 rather than resident cells in the brain and spinal cord (see Figure 1).[82] Also, cranial IR was capable of adequately changing the brain's milieu to allow for the infiltration of peripherally derived, proinflammatory $CCR2^+$ MΦ.[14] Similarly, secretion of tumor necrosis factor alpha (TNF-α) triggered the secretion of CCL2 in astrocytes (see Figure 1).[83] In addition, augmented secretion of CCL2 triggered changes in the integrity of the BBB.[84] Moreover, IR was able to induce anti-inflammatory gene signature in TAMΦ in two spontaneous GBM models.[41]

Notably, the underlying mechanism was mainly via cell death in IR M0 and M1 MΦ in GL261 GBM model.[34,62] Interestingly, TAMΦ phagocytic activity triggered the removal of apoptotic cells in *in vitro* human GBM cell lines following IR (see Figure 1).[34] Moreover, a total of 3% of cells were apoptotic cells following IR exposure to the GBM cell lines.[41] Also, higher phagocytic activity triggered the secretion of higher levels of "eat-me" receptors such as the efferocytosis (the process by which apoptotic cells are removed by phagocytic cells) receptor AXL following MΦ exposed to IR-treated GBM cell conditioned media (see Figure 1).[34] Furthermore, the ability of MΦ to prime T cells was inhibited by IR in GBM.[85] Moreover, IL-8 was detected in necrotic areas of the tumor and around MΦ during immunochemistry analysis in about 50% of the patients with GBM following IR (see Figure 1).[77]

MΦ Induce Radiosensitization and Radioresistance

Radiosensitization is a physical, chemical, or pharmacological agent that augments the lethal effects of IR when administered in conjunction with IR. It was established that M1 MΦ are more sensitive to IR than M2 MΦ.[62] Notably, temozolomide (TMZ) is an oral alkylating agent used to treat malignant glioma such as GBM and astrocytomas.[34] Also, concurrent administration of IR and TMZ augmented the phagocytic activity of MΦ against four different human GBM cell lines (see Table 1).[34] Similarly, the MΦ modulated the paracrine effect of GBM cells exposed to IR as well as TMZ and this correlated with the percentage of apoptotic GBM cells (see Table 1).[34]

It is worth noting that the positive correlation between MΦ phagocytic activity and the stimulation of apoptosis in GBM cells by IR and TMZ suggests that apoptotic cells are critical in the modulation of phagocytic activity, whereas necrotic as well as secondary necrotic cells does not.[34] Also, IR facilitated a more immunosuppressive milieu via the stimulation of efferocytosis in TAMΦ and an upsurge of tumor cell engulfment by TAMΦ exhibited detrimental effect of the anti-tumoral immune response in GBM.[34] Similarly, NO was able to stimulate radiosensitization in GBM cells under hypoxic conditions by enhancing DNA DSBs, blockade of DNA repair, as well as activation of mitotic catastrophe via TAMΦ (see Table 1).[86,87]

It is well established that SDF-1/CXCL12 is able to trigger glioma invasion by recruiting MΦ or T-regulatory cell to the peritumoral area, via initiation of interaction between endothelial cells or by marshaling hematopoietic stem cells as well as progenitor cells.[89] Also, plerixafor (AMD3100) is a macrocyclic compound that is able to irreversibly antagonize against the binding of CXCR4 with its ligand SDF-1/CXCL12 (see Table 1).[66,89] Furthermore, the blockade of the cross-talk between SDF- 1 with its receptor, CXCR4, by AMD3100 augmented the effectiveness of IR in GBM (see Table 1).[66]

Programmed cell death protein 1 (PD-1) blocks immune responses as well as facilitates self-tolerance via the activation of

Table 1. Show the influential effects MΦ induce radiosensitization and radioresistance agents and the mechanisms via which they trigger these effects.

AGENT	INFLUENCE VIA MΦ	IR-INDUCE MECHANISM OF ACTION VIA MΦ	CITATION
TMZ	Radiosensitization	IR and TMZ augmented the phagocytic activity of MΦ against four different human GBM cell lines.	Paolicelli et al[31]
		MΦ modulated the paracrine effect of GBM cells exposed to IR and TMZ via apoptosis	Paolicelli et al[31]
NO	Radiosensitization	NO and IR enhancing DNA DSBs, blockade of DNA repair and activation of mitotic catastrophe via TAMΦ in GBM	Semple et al,[83] Roberts et al[84]
AMD3100	Radiosensitization	Inhibition of cross-talk between SDF- 1 and CXCR4 by AMD3100 augmented the effectiveness of IR in GBM	Heckler et al[86]
PD-1	Radiosensitization	PD-L1$^+$ circulating monocyte-derived MΦ are the cells that respond primarily to IR sensitivity during GBM therapy	Lomax et al[87]
		AntiPD-L1 was able to directly stimulate PD-L1$^+$ MΦ to augment production of cytokines and increase phagocytosis in an ERK signaling-dependent fashion following IR	Lomax et al[87]
SDF-1/ CXCL12	Radioresistance	An upsurge of SDF-1α at the tumor invasion front after IR was correlated with the recruitment of TAMΦ as well as radioresistance in a murine glioma model.	Takenaka et al[76]
		SDF-1/CXCL12 triggers glioma invasion by recruiting MΦ or T-regulatory cell to the peritumoral area, via initiation of interaction between endothelial cells or by marshaling hematopoietic stem cells as well as progenitor cells	Tseng et al[85]
Mesenchymal cells state	Radioresistance	TAMΦ induces cell differentiation in GBM to a mesenchymal state via generated NF-κB following IR	Miyamoto et al,[30] Wunderlich et al[88]
VEGF	Radioresistance	TAMΦ subtypes and a higher microvascular density associated with higher levels of VEGF receptor-1 (VEGFR-1) were observed following IR in glioma	Takenaka et al[76]

Abbreviations: AMD3100, plerixafor; CXCL12, cyclooxygenase-2; CXCR, chemokine receptor; ERK, extracellular signal-regulated kinase; GBM, glioblastoma multiforme; IR, ionizing radiation or irradiation or radiotherapy; MΦ, macrophages; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NO, nitric oxide; PD, programmed cell death; SDF, stromal cell-derived factor; TAMΦ, tumor-associated macrophages; TMZ, temozolomide; VEGF, vascular endothelial growth factor.

T cells, apoptosis of antigen-specific T cells as well as blockade of apoptosis of T-regulatory cells. Interestingly, PD-L1$^+$ circulating monocyte-derived MΦ are the cells that respond primarily to sensitivity IR during GBM therapy (see Table 1).[90] Notably, after IR, mouse with GBM responded better to anti-PD-L1 therapy, which precisely target infiltrating PD-L1$^+$ MΦ, than to anti-PD-1 immunotherapy.[91] Thus, patients with GBM that fail anti-PD-1 or anti-PD-L1 monotherapy can still respond to anti-PD-L1 combined with high-dose IR of viable tumor cells. Mechanically, antiPD-L1 was able to directly stimulate PD-L1$^+$ MΦ to augment production of cytokines as well as increase phagocytosis in an ERK signaling-dependent fashion following IR (see Table 1).[90]

Radioresistance is a process in which the tumor cells or tissues adapt to the IR-induced changes and develop resistance toward the IR. Notably, MΦ inside the tumor mass are associated with multiple phenomena that include IR resistance. Paradoxically, although some studies have described MΦ as a radioresistant cell type,[71,88] other studies detected either an upsurge in MΦ in the tumor following x-IR or a reduction.[70] However, MΦ facilitate tumor development as well as represent a negative prognostic factor due to the occurrence of M2 MΦ.[56,92] Also, IR triggers changes in the tumor milieu resulting in tumor aggressiveness and recurrence typically occurring near the IR area.[93]

It is worth noting that IR triggered a quick inflammatory response resulting in TAMΦ recruitment at tumor milieu and this inflammatory response correlated with a short survival time.[77] Moreover, TAMΦ were capable of inducing cell differentiation in GBM to a mesenchymal state via generated NF-kB, and this correlated with IR resistance (see Table 1).[94] Furthermore, a positive modulation of MΦ chemotaxis, which triggered radioresistance in GBM model was detected via total RNA sequencing following IR exposure.[95]

As indicated early, the response of TAMΦ to IR-induced milieu changes in the primary tumor core and the tumor-invading front were different though IR augmented the TAMΦ density in both the primary tumor core as well as the tumor-invading front.[20,65] Also, the invading tumor front has a distinct milieu from that of the primary tumor core in ALTS1C1 tumor model because a different ratio of the TAMΦ subtypes and a higher microvascular density associated with higher

levels of VEGF receptor-1 (VEGFR-1; see Table 1) and SDF-1 secretion were observed following IR.[79] Moreover, an upsurge of SDF-1α at the tumor invasion front after IR correlated with the recruitment of TAMΦ as well as radioresistance in a murine glioma model.[79]

Notably, exposure of IR to the tumor induced modification of multiple pathways and triggered changes in the MΦ activation type, making them more supportive of tumor growth.[96] Also, an increase in M1 MΦ augmentation score correlated with a poor prognosis in GBM patients following IR during a subgroup analysis.[96] Thus, GBM patients with elevated M1 MΦ infiltration had a poorer survival rate. In addition, the subgroup analysis revealed that MΦ were more augmented in IDH-mutant patients, compared to IDH-wild type patients.[97] Thus, GBM patients with IDH mutation had a better survival. Moreover, M1 MΦ had unfavorable prognosis following IR in IDH-wild type GBM patients.[96] Thus, in GBM, M1 MΦ targeted therapies are potential sensitization for IR.

MΦ and IR-Induced Abscopal and Bystander Effects

The abscopal effect occurs when IR shrinks the targeted tumor as well as untreated tumors elsewhere in the body. Thus, any substantial enhancement in survival for GBM patients will necessitate support from the immune system to kill resistant/residual tumor cells outside prior treatment regions.[90] It is worth noting that specific mechanism associated with the abscopal phenomenon remains a paradox. However, IR exposure to tumor triggers a systemic immune response to un-IR and distant tumor foci.[98] Remarkably, the relative influence of antigen-presenting cells (APCs) such as MΦ, dendritic cells, or T cells to the abscopal response differs depending on type of IR, dosage, animal model, as well as immune checkpoint blockers.[99]

The IR-induced tumor death triggered the secretion of neo-antigens or neo-epitopes, which were engulfed by APCs prior to T-cell stimulation during the abscopal response.[100] Furthermore, APCs subsequently entered and circulated then to lymph nodes where neo-antigen presentation triggered the stimulation as well as education of naïve T cells.[90] Moreover, stimulated as well as tumor-specific T cells join the circulation and selectively target tumor cells, leading to regression of un-IR tumors.[90] Notably, GBM with neo-epitopes such as epidermal growth factor receptor variant III (EGFRvIII) tumors were more susceptible to immune-related abscopal response[90] and direct stimulation of MΦ was associated with the abscopal response in the absence of T-cell infiltration.[101]

The IR-induced bystander effect (IRIBE) is the phenomenon in which non-IR cells show effects along with their different intensities due to signals received from nearby IR cells.[70,102] Notably, following WBRT, an IRIBE is often observed in the blood vessels and blood components because the brain is vascularized and contain blood. Interestingly, IRIBE is capable of triggering a sequence of biological endpoints such as augmented micronuclei formation, sister chromatid exchanges, carcinogenesis, as well as decreased cell survival.[102] Thus, IR-induced blood injury is a critical health risk to glioma patient receiving IR.

Specifically, lymphocytes and MΦ are two key constituents in the blood that interrelate with each other as well as influence body organs via blood flow.[70] Notably, MΦ are crucial triggers of bystander signaling factors, and are therefore critical in IRIBE following IR exposure to tissues.[103] Also, lymphocytes are exceedingly sensitive cells that frequently interact with neighboring MΦ in the body following IR.[104] It has been speculated that MΦ are refined by tumor antigens released following IR resulting in a tumor-specific response.[70]

Interestingly, infiltrating MΦ are differentiated by anti-PD-L1 antibodies into anti-tumor states resulting in suppression of previously viable tumor cells just outside of the IR area following IR-induced their recruitment in brain.[90] Also, the effect of anti-PD-1 was not similar to anti-PD-L1 following IR exposure to GBM tumor model.[90] Furthermore, MΦ are resistant to inhibition of metabolic activity by IR with low energy carbon ions. However, there were no differences in the consequences of equivalent doses of x-IR or carbon ions on MΦ as measured in the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolimbromid (MTT) test after IR.[71,105]

It is worth noting that over-secretion of manganese superoxide dismutase (MnSOD) facilitated resistance of cells as well as tissues to IR.[105,106] Also, MnSOD–plasmid–liposome complexes triggered substantial protection of cells and tissues from IR damage.[105] Furthermore, over-secretion of the MnSOD transgene triggered an unexpected upsurge in tumor cell toxicity as well as radiosensitization via the generation of H_2O_2 by the action of MnSOD.[107] Interestingly, MΦ were capable of generating MnSOD in response to IR and this preserved the cells from the harmful effects of IR-induced radical.[108]

Also, MnSOD was induced by various agonists such as lipopolysaccharides (LPS), proinflammatory cytokines, hypoxia, and IR.[71] Notably, the generation of MnSOD was triggered by IR resistance.[71] Similarly, IR of RAW 264.7 MΦ alone with either x-IR or carbon ions did not trigger the generation of the proinflammatory cytokines such as TNF-α and IL-1β or the ROS and NO.[71] Moreover, stimulation of murine as well as rat MΦ triggered iNOS generation via several substances such as LPS, IL-1β, IL-6, IFN-γ, TNF-α, or by oxidative stress, whereas human cells require a multifaceted cytokine amalgamation for iNOS stimulation following cranial IR.[71,109]

Conclusions and Perceptive

Notably, cranial-IR is capable of inducing the migration of peripherally derived MΦ into the brain parenchyma. Moreover, acute response to IR exposure was responsible for the induced peripheral MΦ immigration into the brain. Also, MΦ inside the tumor mass are associated with multiple

phenomena that include IR resistance and enrichment of the M2 MΦ after IR is able to facilitate GBM recurrence. In addition, MΦ are crucial triggers of bystander signaling factors and, are therefore critical in IRIBE following IR exposure to tissues. Therefore, future studies ought to focus on the biomarker role of peripheral MΦ following cranial-IR therapy for glioma.

TAMΦ were not brain-resident microglia, but mainly monocyte-derived MΦ from peripheral blood in GBM milieu. IR facilitated a more immunosuppressive milieu via the stimulation of efferocytosis in TAMΦ and an upsurge of tumor cell engulfment by TAMΦ exhibited detrimental effect of the antitumoral immune response in GBM. In addition, IR exposure to tumor triggers a systemic immune response to un-IR and distant tumor foci in GBM. Furthermore, IR triggered a quick inflammatory response resulting in TAMΦ recruitment at tumor milieu and this inflammatory response correlated with a short survival time. In GBM, M1 MΦ targeted therapies are potential sensitization for IR. Therefore, future glioma therapeutic agents ought to include agents that are capable of altering the TAMΦ in glioma.

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