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miRNA-based therapeutic potential of stem cell-derived extracellular vesicles: A safe cell-free treatment to ameliorate radiation-induced brain injury

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Abstract

Purpose: This review compiles what is known about extracellular vesicles, their bioactive cargo, and how they might be used to treat radiation-induced brain injury. Radiotherapy (RT) is effective in cancer treatment, but can cause substantial damage to normal central nervous system tissue. Stem cell therapy has been shown to be effective in treating cognitive dysfunction arising from RT, but there remain safety concerns when grafting foreign stem cells into the brain (*i.e.* immunogenicity, teratoma). These limitations prompted the search for cell-free alternatives, and pointed to extracellular vesicles (EV) that have been shown to have similar ameliorating effects in other tissues and injury models.

Conclusions: EV are nano-scale and lipid-bound vesicles that readily pass the blood-brain barrier. Arguably the most important bioactive cargo within EV are RNAs, in particular microRNAs (miRNA). A single miRNA can modulate entire gene networks and signalling within the recipient cell. Determining functionally relevant miRNA could lead to therapeutic treatments where synthetically-derived EV are used as delivery vectors for miRNA. Stem cell-derived EV can be effective in treating brain injury including radiation-induced cognitive deficits. Of particular interest are systemic modes of administration which obviate the need for invasive procedures.

Keywords

extracellular vesicles; stem cells; cognitive function; miRNA; radiotherapy

Introduction

While survival is rightfully considered the primary criteria for successful cancer treatment, increased success in oncology translates into an increasing population of survivors suffering from unintended side effects of treatment. A significant number of patients surviving more than six months post radiotherapy (RT) suffer cognitive impairments that impact quality of life. These decrements are debilitating, persistent and progressive, and are especially problematic in pediatric patients (Roman & Sperduto 1995; Abayomi 1996; Anderson et al.

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Declaration of interest

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2000). These studies suggest that neurocognitive endpoints should be considered a major criteria for successful therapeutic outcome. So what is the etiology and pathology of these problems associated with RT? While effective in solid tumor treatment and prevention of metastasis to the CNS, RT also causes brain injury - ranging from acute (within days to weeks), to early delayed (1–6 months), to late delayed (6+ months) post-RT. Acute and early side-effects such as nausea, vomiting, and headaches can be managed, but the late delayed side-effects such as intellectual impairment, memory loss, and dementia are usually irreversible. The pathogenesis of radiation-induced cognitive impairment is very complex and not fully understood, but the current model suggests a combination of persistent oxidative stress (Robbins et al. 2002), chronic inflammation (Hong et al. 1995; Rola et al. 2005; Ramanan et al. 2008), demyelination (Nagesh et al. 2008), morphometric degradation of neurons (Burger et al. 1979; Parihar et al. 2015), inhibition of neurogenesis (Madsen et al. 2003; Rola et al. 2004; Manda et al. 2009), disruption of neurogenic niche signaling (Monje et al. 2002) and angiogenesis (Warrington et al. 2013) that hinders hippocampal and non-hippocampal-dependent learning, memory, and spatial information processing (reviewed in (L. Zhang et al. 2015)). These phenomena combine and interact to create conditions that are remarkably similar to aging, and in addition, to those seen in progressive degenerative disorders such as Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, highlighting the extensive overlapping pathology between neurodegenerative diseases and cranial irradiation, where resultant cognitive deficits impact the memory of 40–50% of surviving patients (Meyers 2000). Despite improvements in technology and technique, standard of care treatments continue to induce cognitive impairments in survivors. Promising innovations in immune therapy and gene editing aside, development of clinical resources to remediate the radiation-injured brain remains a critical priority for the growing numbers of affected patients suffering from treatment-associated toxicities. This review explores the possiblity that stem cell derived extracellular vesicles might just represent that therapeutic candidate.

Therapeutic strategies to ameliorate radiation-induced brain injury

Ever since their discovery, stem cells have captured the imagination, holding great potential for regenerative medicine. The term stem cell is broad and includes pluripotent embryonic stem cells (ESCs), and multipotent derivatives including mesenchymal stem cells (MSCs), neural stem cells (NSCs), hematopoietic stem cells (HSCs) and various progenitor cells from all over the body. ESCs are fully pluripotent and can differentiate into any type of tissue. MSCs, NSCs, and HSCs are multipotent and therefore partially lineage-limited, able to differentiate into bone/cartilage/muscle/fat, neural cells, and blood cells, respectively. As a renewable source of undifferentiated cells, able to continuously grow and divide, it was shown that they could be transplanted to a new host or location and the new environmental cues would stimulate the cells to differentiate (Shihabuddin et al. 2000) to replace and repopulate the damaged tissue (reviewed in (Benderitter et al. 2014)). Unfortunately this simple and idealistic view has generally not borne out experimentally, with the notable exception of bone marrow transplantation (*i.e.* HSC transplantation), used to replenish the hematopoietic system after ablative radio/chemotherapy. However, there is promising research in a number of areas using stem cell therapy including: stroke (Bang et al. 2005; Savitz et al. 2011), severe burns (Lataillade et al. 2007), rheumatoid arthritis (Gonzalez-Rey

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et al. 2009; González et al. 2009), myocardial infarction (Meyer 2006; Schächinger et al. 2006; Lunde et al. 2006; Zhang et al. 2009), hearing loss (Li et al. 2004; Zhou et al. 2011), retinal disease (Meyer et al. 2011; Ng 2014), and neurodegenerative diseases (Lindvall et al. 2004; Joyce et al. 2010). Given the nature and scope of RT-induced side effects (*e.g.* normal tissue damage surrounding tumors) in the brain, cognitive deficits represent an adverse condition primed for stem cell therapy.

Indeed, it has been demonstrated that stem cell-based therapies can be effective in treating physical brain or spinal cord injury in rodents (Chopp et al. 2000; Tsuji et al. 2010). Radiation exposure has been well established to deplete neural stem cell populations, and previous efforts led by Dr. Limoli's group employed NSC therapy to treat radiation-induced cognitive deficits. Specifically, athymic nude (ATN) rats received intra-hippocampal transplantation of human neural stem cells (hNSCs, H9 derived) or induced pluripotent stem cells (iPSC) after cranial irradiation (Acharya et al. 2011; Acharya et al. 2014; Acharya et al. 2015). The stem cell treated rats performed consistently better over a battery of behavioral tests compared to irradiated rats receiving vehicle. In addition, the neuronal structures were preserved, and the host hippocampus had less neuroinflammation as measured by microglial activation. A small percentage of the grafted stem cells were also shown to integrate into the host hippocampal circuitry. While these data are very promising, there are issues associated with stem cells therapies. Beyond the ethical concerns, other risks include teratoma formation and immunorejection (Bradley et al. 2002; Chopp & Zhang 2015). To avoid immunorejection, rodent studies relied on the use of immunocompromised hosts (Acharya et al. 2011). In human patients receiving non-self stem cells, immunosuppression would be necessary and can be problematic since the long-term use of immunosuppressants can result in toxicity, particularly in aged individuals (Mollison et al. 1998). To address these critical issues associated with stem cell therapies, researchers have been looking for safer and more efficacious alternatives.

Extracellular vesicles

Extracellular vesicles (EV) were originally thought to be a "disposal mechanism" for cellular garbage (Pan & Johnstone 1983), but have now been shown to be important both for cell-to-cell communication and microenvironment maintenance (Théry 2011). This autocrine/paracrine signaling mechanism is now considered a short or long distance mode of communication common to most all cells and tissues of the body. The classification of various EV is of some debate, however it is generally accepted that these membrane-bound vesicles are divided into two groups based on size and mode of formation. Microvesicles (MV) tend to be larger - ranging from 100 nm to 1 μm - and are created by direct assembly and outward budding from the cell membrane (Bucki et al. 1998). External stimuli such as hypoxia or the influx of Ca^{2+} can trigger the release of MV from the cell (Bucki et al. 1998). The biogenesis of exosomes which tend to be smaller - 30 to 100 nm - involves the release of intraluminal vesicles contained inside the endosome-derived multivesicular body (MVB) by fusion with the plasma membrane (György et al. 2011). During this process, the bioactive cargo from the cytosol is sorted into the tiny vesicles. MVB formed by this mechanism release exosomes into the extracellular space when they fuse with and bud off from the

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plasma membrane (Cocucci & Meldolesi 2015). The release of exosomes is known to involve Rab GTPases (Abels & Breakefield 2016).

EV are secreted by cells throughout the body both in normal physiological conditions and diseased states including cancer. Because they are found and readily characterized in a wide range of bodily fluids including blood, urine and cerebrospinal fluid (Raposo & Stoorvogel 2013) EV are of tremendous interest as circulating biomarkers of disease or exposure (Luga et al. 2012; Frühbeis et al. 2013). Importantly, EV have low immunogenicity, a long half-life in circulation, and are able to cross the blood-brain barrier (Alvarez-Erviti et al. 2011; Kalani et al. 2013; Zhu et al. 2017). While further study is needed to confirm a lack of immunogenicity or off target effects, these features further bolster enthusiasm for the use of EV, not just as biomarkers, but also as promising therapies for regenerative medicine. While extensive long-term studies have yet to be completed, evidence suggests that EV therapy will be well tolerated. Macrophage derived EV, loaded with catalase were administered to mice every other day for a total of 10 treatments and were well tolerated and effective in reducing Parkinson's Disease related neuroinflammation in mice (Haney et al. 2015). Similarly, daily curcumin-loaded EV therapy for 31 consecutive days caused no adverse side effects and was effective in three mouse models, reducing LPS-mediated neuroinflammation, auto-immune encephalomyelitis, and also in delaying brain tumor growth (Zhuang et al. 2011).

EV Uptake

EV travel through the extracellular space to nearby cells or even through circulation to distant cells. It is thought that EV are able to identify target cells using interactions between transmembrane proteins on the EV and specific receptors on the surface of the target cell. Recipient cells internalize EV via either fusion with the plasma membrane or by endocytosis (Mulcahy et al. 2014). One factor that seems to be important for EV uptake is surface heparin sulfate proteoglycans (HSPGs). It has been shown that blocking HSPGs decreases EV uptake by target cells (Atai et al. 2013). While direct entry can be achieved by membrane fusion, the most common method of EV uptake is through endocytosis. Despite the fact that this pathway generally leads to degradation or re-export, functional transfer of nucleic acids has been demonstrated both *in vitro* and *in vivo*. Intact transfer of functional nucleic acids may be directed by a cell-specific ligand/receptor interaction in specific target cell types.

EV Cargo

EV cargo can include proteins, lipids, mitochondria, and nucleic acids. It has been found that bioactive cargo is responsible for cell-to-cell signaling and environmental responses (Raposo & Stoorvogel 2013). The membrane protects EV contents from extracellular proteases and nucleases (Théry et al. 2002). EV have been readily isolated from serum-free conditioned cell culture media using a number of different techniques including differential centrifugation, sucrose gradient centrifugation, microfiltration, immune-affinity capture, microfluidics devices, polymer based products such as ExoQuick™, and size-exclusion liquid chromatography (Momen-Heravi et al. 2013; Nordin et al. 2015). Quantification of EV can be achieved using nanoparticle tracking instruments (*e.g.* Nanosight and Zetaview)

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that utilize Brownian motion to determine concentration and size distribution of EV samples. Once isolated, EV have been shown to be stable and biologically active for 20 months at 4°C (Kumeda et al. 2017) and likely even longer at −80°C. Due to variations in EV isolation protocols and analysis, characterization of EV subtypes and the bioactive cargo within has been difficult. However, given the promise of EV-based therapies a significant a concerted effort is being made to refine isolation protocols, and to identify and catalog the various types of cargo found within EV from a variety of cell types. These data are conveniently searchable in online databases including Exocarta (Keerthikumar et al. 2016), Vesiclepedia (Kalra et al. 2012), and EVpedia (Kim et al. 2015) and include purification procedures for reproducibility and consistency. Further refinement and standardization of isolation methods of EV remains, for now, a techological challenge to the advancement of EV therapies, as does the characterization of EV cargo and understanding the function of those cargo in targeting specific recipient cells and effecting phenotypic changes in those cells following uptake.

Proteins

In general, the most common proteins found in EV are involved in the packing, biogenesis, and release of the EV themselves. EV generally have tetraspanins - namely CD63, CD81, and CD9 - and other transmembrane proteins such as LAMP1. Other common proteins include those involved in signal transduction and antigen presentation (Abels & Breakefield 2016).

Lipids

Lipid content of EV varies, but usually mimics the lipid content of the cell type from which the EV are derived. However, some lipids are specifically associated with EV types. Sphingomyelin, cholesterol, ganglioside GM3, disaturated lipids, phosphatidylserine, and ceramide are enriched in EV (Llorente et al. 2013), while phosphatidylcholine and diacyl-glycerol are depleted (Laulagnier et al. 2004).

Mitochondria

Mitochondrial components (including DNA) have been described in EV, though little is known about how the phenomenon occurs. Based on the size and capacity of the EV, either mitochondrial fragments/proteins and DNA in smaller EV (Guescini et al. 2009) or full functional mitochondria in larger EV (Hayakawa et al. 2016) have been observed. As such, EV are known to participate in mitochondrial transfer whereby mitochondrial components or full mitochondria are horizontally transferred between cells (Torralba et al. 2016). Whether the mitochondrial content of certain EV contribute to the regenerative properties of stem cells has yet to be determined.

Nucleic acids

In a minority of cases, some DNA has been found in EV, namely genomic and mitochondrial DNA. To date, there is little evidence for EV-mediated horizontal gene transfer in normal cells though, but rather that transfer of EV-DNA might play a role in intercellular communication by cancer cells in the cancer microenvironment or in metastasis. It has been

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shown that normal cells have cellular defense mechanisms that prevent the delivery and integration of EV-DNA into the genome of those normal cells (Kawamura et al. 2017). However, an overwhelming majority of the nucleic acid material in the EV is RNA. Many different types of RNA have been found in EV, including: mRNAs, microRNAs (miRNAs), rRNAs, long and short non-coding RNAs (ncRNAs), tRNA fragments, piwi-interacting RNA, vault RNA, and Y RNA (Abels & Breakefield 2016). For the most part the fragments of RNA are limited to 200 bp with a small portion extending to up to 4 kB indicating that most mRNAs and long ncRNAs are fragmented (Batagov & Kurochkin 2013). In fact, the mRNAs are thought to play more of a regulatory role, attracting specific miRNAs to the EV, than a functional one. However, it has also been shown that full-length mRNAs transferred by EV can be readily transcribed in the recipient cell (Valadi et al. 2007).

MicroRNAs

MiRNAs are a family of non-coding RNAs ranging between 20 and 25 bases in length that are able to affect protein levels by post-transcriptional regulation of messenger RNAs (mRNAs). miRNAs are small single-stranded RNAs that compliment the 3’ UTR of mRNA and are able to shut down the translation of the mRNA transcript as a part of the RNA-induced silencing complex (RISC) (Rana 2007). In addition, RISC-mediated silencing promotes deadenylation which hastens the degradation of mRNA transcripts. miRNAs have been discovered to be important in the brain and CNS in both healthy and disease states. One such miRNA, miR-124, is the most highly enriched in the brain and has been shown to be important for adult neurogenesis (Makeyev et al. 2007; Cheng et al. 2009). Another miRNA, miR-125a, has been shown to downregulate synthesis of postsynaptic density protein 95 (PSD-95) (Muddashetty et al. 2011), which is elevated in the irradiated hippocampus (Chmielewski et al. 2016). Replenishing or restoring normal miRNA levels has been suggested as a method for treating various types of brain injury.

miRNA as Active EV Cargo

Based on the evidence available to date, miRNAs are considered to be critical functional elements of EV (J. Zhang et al. 2015). In general, the miRNA contents of the EV match the contents of the cytoplasm of the cells from which they are derived, with some exceptions. It has been shown that miRNA overexpression in the EV-producing cell led to an increased level of the miRNA in EV, while miRNA depletion causes a decreased level in EV (Squadrito et al. 2014). However, there is evidence of selection in terms of which miRNAs are loaded into the EV. There are a four known mechanisms for this that suggest that the selection process is very complex and tightly regulated.

1. GGAG motif - a binding site for specific ribonucleoprotein thought to be involved in EV loading (Villarroya-Beltri et al. 2013)
2. 3’ end uridylation - post-transcriptional modification that appears to contribute to sorting miRNAs into EV (Koppers-Lalic et al. 2014)
3. nSMase2 route - overexpression leads to more exported miRNAs (Kosaka et al. 2010)

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4. RISC/AGO2 association - RISC components are also involved in targeted loading of EV (Guduric-Fuchs et al. 2012)

miRNAs are stable, transferred and delivered intact, and are able to change the target cell phenotype and/or physiology, influencing not just one gene, but entire cellular pathways or signaling cascades (Baumann & Winkler 2014). This can be done through two modes, the more canonical silencing RNA (siRNA) pathway by which the miRNA can bind to sites on mRNA and silence translation, and a more recently discovered ability to act as a Toll-Like Receptor ligand and activate immune cells (Fabbri et al. 2012). It is even possible for overexpressed exogenous miRNAs to get loaded into EV (Pegtel et al. 2010), leading to exciting possibilities for EV-mediated delivery of therapeutic miRNAs to a targeted cell population. In fact, targeted mesenchymal stem cell-derived EV loaded with exogenous miR-124 have been used to promote neurogenesis following stroke in mice (Yang et al. 2017). Further, mir-199a-laden EV were able to increase proliferation and decrease apoptosis in cardiomyocytes *in vitro* (Ferguson et al. 2018). That same group also loaded EV with miR-130a-3p and demonstrated an increase in all angiogenic endpoints in HUVECs. Examples of EV-associated miRNAs that have been isolated from specific cell types and have known biological effects in target cells can be found in Table 1. It will be essential that these engineered EV be carefully tested for off-target effects since it is possible, depending on the mode of delivery, that the these EV are introduced to cells that were not the intended target. However, the Yang et al. (2017) study used a specific targeting peptide to ensure fusion with target cells. Despite the caveats to this approach, these data strongly support the hypothesis that miRNAs are functional important cargo of EV able to exert profound effects in recipient cells.

EV as Therapeutic Agents in Regenerative Medicine

EV Therapy for Radiation Induced Cognitive Deficit

As alternatives to stem cell therapy, studies have found that stem cell-derived EV can provide equivalent beneficial effects compared to stem cells themselves in damaged tissues without the risk of teratoma formation (Doeppner et al. 2015). In inflammation-induced and physical injury-induced cases of cognitive dysfunction in rodents, mesenchymal stem cell-derived EV have reduced inflammation and increased performance in behavioral testing (Y. Zhang et al. 2015; Drommelschmidt et al. 2017; Zhang et al. 2017). Indeed, the Limoli lab has engrafted EV as opposed to the hNSCs from which they were derived (Baulch et al. 2016). The results were striking. Similar to engrafted hNSC, administration of EV ameliorated the adverse effects of cranial irradiation, as indicated by improved cognition, reduced inflammation, and preserved neuronal architecture (Figure 1). Further, the reduced inflammation in not only the hippocampus, but also in the cortex and the amygdala suggested that EV grafting could exert significant effects in regions of the brain both proximal and distal to the engraftment site. Combined with the previous study (Acharya et al. 2011) that showed that only 12–15% of grafted hNSCs remained four months after transplantation surgeries and that out of those remaining cells, less than a quarter of them had differentiated into neuronal lineages, these data suggest a trophic support role of engrafted stem cells is more likely than one of cell replacement. However, the EV study was performed in immunocompromised rats as opposed to wild-type animals. Clearly, proof-of-

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principle studies need to be carried out in wild-type, immunocompetent animals to define any adverse immune response of human NSC-derived EV therapy.

Systemic Administration of EV

A newer area of research is systemic administration of therapeutic EV. The capability of EV to readily cross the blood-brain barrier (Kalani et al. 2013), opens a number of opportunities for systemic delivery of EV, thereby avoiding invasive surgical procedures. Methods of systemic administration include intranasal, intraperitoneal, retro-orbital sinus, and tail vein injections (*e.g.* intraveneous or IV). These relatively new strategies have been used successfully for a number of different conditions including stroke (Xin et al. 2013), traumatic brain injury (Y. Zhang et al. 2015), and myocardial infarction (Timmers et al. 2011; Barile et al. 2014; Zhao et al. 2015; Khan et al. 2015). More specifically, intranasal administration of EV has been successful in treating Parkinson's Disease (Haney et al. 2015), nasal allergies (Prado et al. 2010), stroke (Kalani et al. 2016), epilepsy-related brain damage (Long et al. 2017), and lung injury (Rice et al. 2017; Tan et al. 2018). In addition, the previously mentioned EV-mediated delivery of miR-124 post-stroke utilized tail vein injections (Yang et al. 2017). The potential benefits of these methods should be readily apparent - bypassing the need for invasive heart, brain, or lung surgical procedures to treat patients. In the context of treating the radiation injured brain, intranasal treatment represents a more direct delivery to the brain, bypassing peripheral organ filtration or dilution that occurs via IV administration, promoting a more rapid and direct delivery of EV to the brain (Haney et al. 2015). While gaps in knowledge remain to be closed regarding EV, the minimal immunogenicity, lack of teratoma concerns, and potential for systemic administration, therapeutic stem-cell derived EV represent a very promising direction for the treatment of radiation-induced cognitive dysfunction, in addition to many other side effects of radiation exposure.

Conclusion and Future Perspectives

The use of EV represent an emerging and innovative cell-free approach for the treatment of a variety of adverse conditions without the need for immunosuppression. Such approaches will likely include modified biological EV or synthetically manufactured EV loaded with specific therapeutic miRNAs. Ultimately, these therapies will help an underserved population of cancer survivors, desperately in need of novel interventions designed to minimize normal tissue complications and improve quality of life following RT.

It is possible and perhaps inevitable that in the future, EV-based therapies will be used to treat more than just radiation-induced cognitive deficits. Other potential targets could include traumatic brain injury, age-related memory loss, and neurodegenerative diseases. Eventually, it may even be possible to specifically tailor EV therapy to protect first responders to a radiologic accident such as Fukushima, victims of a terrorist mediated nuclear attack, or even a space traveler over the course of a deep space mission to Mars. The potential of this technology to resolve the adverse effects of radiation exposure, as well as other adverse indications, is extremely exciting and the future of the field looks bright.

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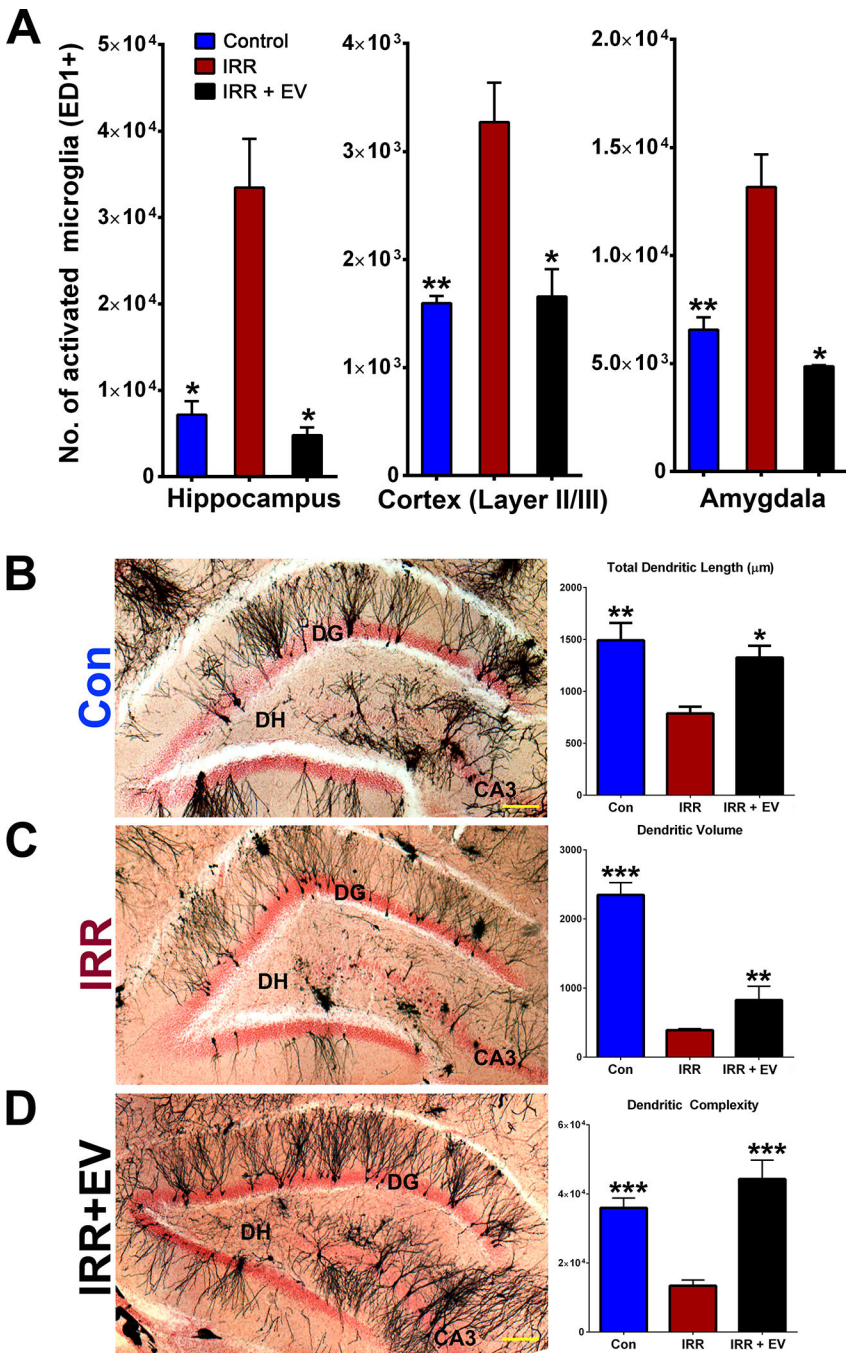

Figure 1.

EV treatment in cranially-irradiated athymic nude rats ameliorated radiation-induced neuroinflammation and damage to neuronal structure. (A) Immunohistochemical identification and stereology quantification of activated microglia showed that, compared with controls, irradiation significantly increased the number of activated microglia in all regions of the brain evaluated. Compared with the irradiated (IRR) cohort, IRR+EV animals had significantly lower numbers of activated microglia in the hippocampus, cortex (layer II/III), and amygdala. (B-D) Representative images of Golgi–Cox-impregnated hippocampal

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tissue sections from Control (Con), IRR, and IRR+EV illustrate the gross disruption of neuronal structure (black) in the dentate gyrus, dentate hilus and CA3 regions of the hippocampus (DG; nuclear fast red counterstained) after cranial irradiation that is resolved in animals receiving EV. Structural parameters of dendritic morphology (length, volume, complexity) quantified in each cohort demonstrate that radiation-induced reductions in dendritic morphology were ameliorated by EV grafting. Data are presented as mean ± SEM (n = 3–4 rats per group). *$P \geq 0.05$, **$P \geq 0.01$, ***$P \geq 0.001$ (ANOVA and Bonferroni's multiple comparisons test). [Scale bars, 50 μm (B–D).] [adapted from (Baulch et al. 2016)].

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Table 1.

miRNAs associated with EV from specific cell types and known functions based on peer-reviewed literature.

MicroRNA ID	Cells Derived From	Biological Outcome in Target Cells	Reference
let-7 Family	Multiple Types	Suppresses oncogenes and cell-cycle regulators	(Kumar et al. 2008)
miR-19a	Astrocyte	Suppresses tumor suppressor PTEN	(L. Zhang et al. 2015)
miR-21	CPC	Protects myocardial cells against oxidative stress-related apoptosis	(Xiao et al. 2016)
miR-124	CNS cells	Promotes neural differentiation	(Yang et al. 2017)
miR-130a-3p	MSC	Increases angiogenesis	(Ferguson et al. 2018)
miR-132	CPC	Enhances tube formation in epithelial cells	(Barile et al. 2014)
miR-133b	MSC	Promotes neurite outgrowth Recovers brain function after stroke	(Xin et al. 2012; Xin et al. 2013)
miR-199a	MSC	Cardiomyocyte proliferation	(Ferguson et al. 2018)
miR-210	CPC Breast Cancer	Inhibits apoptosis Increases angiogenesis	(Barile et al. 2014) (Kosaka et al. 2013)
miR-294	mESC	Increases survival, proliferation, and commitment of CPCs Increases formation, persistence, and proliferation of cardiomyocytes	(Khan et al. 2015)

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Table 2.

Pros and cons of stem cell therapy as compared to stem cell-derived EV therapy

Stem Cell Therapy		EV Therapy	
Pros	**Cons**	**Pros**	**Cons**
Multipotent	Immunogenic (if not self)	Non-immunogenic	No cell replacement
Cell replacement	Possible Teratoma	Cannot form teratomas	Need to produce large quantities *in vitro*
Includes EV	Cannot cross BBB	Reduce inflammation	Consistency is needed in isolation protocol
Reduce inflammation	Brain surgery necessary for grafting	Readily cross BBB	Can be difficult to culture
Repair of neurogenic niche microenvironment	Can be difficult to culture	Can be administered intravenously or intra-nasally	
		Possibly restore neurogenic niche microenvironment	
		Easily isolated from conditioned media	