Extracellular vesicles as a potential therapeutic for age-related macular degeneration

Lorraine L. C. Chow, Ben Mead

Abstract

Age-related macular degeneration is a major global cause of central visual impairment and severe vision loss. With an aging population, the already immense economic burden of costly anti-vascular endothelial growth factor treatment is likely to increase. In addition, current conventional treatment is only available for the late neovascular stage of age-related macular degeneration, and injections can come with potentially devastating complications, introducing the need for more economical and risk-free treatment. In recent years, exosomes, which are nano-sized extracellular vesicles of an endocytic origin, have shown immense potential as diagnostic biomarkers and in the therapeutic application, as they are bestowed with characteristics including an expansive cargo that closely resembles their parent cell and exceptional ability of intercellular communication and targeting neighboring cells. Exosomes are currently undergoing clinical trials for various conditions such as type 1 diabetes and autoimmune diseases; however, exosomes as a potential therapy for several retinal diseases have just begun to undergo scrutinizing investigation with little literature on age-related macular degeneration specifically. This article will focus on the limited literature available on exosome transplantation treatment in age-related macular degeneration animal models and in vitro cell cultures, as well as briefly identify future research directions. Current literature on exosome therapy using age-related macular degeneration rodent models includes laser retinal injury, N-methyl-N-nitrosourea, and royal college of surgeon models, which mimic inflammatory and degenerative aspects of age-related macular degeneration. These have shown promising results in preserving retinal function and morphology, as well as protecting photoreceptors from apoptosis. Exosomes from their respective cellular origins may also act by regulating the expression of various inflammatory cytokines, mRNAs, and proteins involved in photoreceptor degeneration pathways to exert a therapeutic effect. Various findings have also opened exciting prospects for the involvement of cargo components in remedial effects on the damaged macula or retina.

Key Words: age-related macular degeneration; exosomes; extracellular vesicles; miRNA; neuroprotection; photoreceptors; retina; retinal pigment epithelium

Introduction

Retina, photoreceptors, and the retinal pigment epithelium

The retina belongs to the central nervous system and is a thin complex layer of tissues lining the inner wall of the eye. In the retina, light triggers electrochemical responses in photoreceptors, which send signals to the brain to produce a visual image through a process called phototransduction. Photoreceptors play an important role in the detection and absorption of light and have a high turnover of waste products such as photoreceptor discs or tips (Boulton and Dayhaw-Barker, 2001). The retinal pigment epithelium (RPE) regulates nutrients and metabolic waste transport, and functions as an outer blood-retinal barrier that partially filters particle movement between the choroid and subretinal space. These two are just some of the many mechanisms by which RPE function to maintain photoreceptors (Nowak, 2006; Campbell and Humphries, 2012; Nowak, 2014). Photoreceptors have been proven to be more susceptible to damage than other retinal neurons and their loss is a primary characteristic of a series of diseases known as age-related macular degeneration (AMD) (Stone et al., 1999).

Age-related macular degeneration

AMD is one of the leading causes of irreversible vision loss in the world, accounting for 9% of blindness (Wong et al., 2014). In addition, studies have shown that incidences are rising at an alarming rate, especially within the elderly population, with an expected increase to 288 million by 2040 (Wong et al., 2014; Li et al., 2020). AMD consists of both an early (drusen and pigmentary changes) and late stage (geographic atrophy and neovascular AMD). Various risk factors have been identified with major ones being smoking, previous cataract surgery, age, family history, disputable correlations with a dietary habit, sunlight exposure, and lifestyle (Chakravarty et al., 2010; Armstrong and Mousavi, 2015). Despite substantial research dedicated to AMD, its pathogenesis remains poorly understood due to its complex and multifactorial nature. However, research throughout the years has identified the main cellular disease contributions to drusenogenesis (drusen formation causing Bruch’s membrane degeneration), lipofuscinogenesis (photoreceptor degeneration under continuous oxidative stress), local inflammation (immune system activated by drusen), and lastly neovascularization (disbalance between angiogenic and anti-angiogenic factors) seen in the late choroidal neovascularization (CNV) AMD, resulting in Bruch’s membrane degeneration, RPE damage and subsequent photoreceptor cell damage and death (Nowak, 2006, 2014). AMD is a substantial global burden to health services, hence the rising need for further allocation of research and healthcare resources towards novel treatments.

Existing AMD treatments

The treatment option for AMD depends on the stage of the disease. Currently, apart from controversial evidence on antioxidant diet and supplements such as vitamins C & E, beta-carotene, and zinc in prevention and reduction of AMD progression from an age-related eye diseases study (AREDS) study (AREDS-Research-Group, 2001), there is no direct proven treatment for dry AMD.

There are a few approved treatments available for patients with wet AMD, including photodynamic therapy, laser therapy, and the recent vicenial discovery of anti-VEGF injection or a combination of the above treatments. However, despite anti-VEGF injections being the current gold standard and conventional treatment for wet AMD, significant risks can still come with it despite careful handling. Anti-VEGF injections require intensive and frequent administration for lengthy periods with no guarantee for improved vision (Stahl, 2020). The injections also come with high risks of severe systemic and ocular complications per injection, such as endophthalmitis, ocular hemorrhages, inflammation, and retinal detachment (Fletcher and Chong, 2008; Falavarjani and Nguyen, 2013), and specifically in AMD: incidences of RPE tear in anti-VEGF injections (Ahn et al., 2022). Current wet AMD treatments only work through the blocking of CNV growth and vessel leakage reduction; therefore, there is an absence of a true cure and treatments catered to AMD’s multifactorial nature or initial stages of the disease (Rattner and Nathans, 2006).

Exosomes

In the recent decade, exosomes have garnered growing attention due to their promising potential and prospects in pathological research and uses. Exosomes are a nanosized subset of extracellular vesicles (EVs); along
Exosomal Therapy

Exosomes are being actively studied as a potential therapeutic candidate and a potential alternative to cell therapy. Exosomes show supremacy over their parent cell due to various advantages such as quicker diffusive rate to target cells (Kastelowitz and Yin, 2014) and versatility of movement through the blood-brain/retinal barrier (Zagrean et al., 2018; Banks et al., 2020). Furthermore, exosomes solve previously raised issues that arose with cell-based therapy such as the possibility of immune rejection (Hinada et al., 2017). This is evident with the several several kinds of preparation receiving stem cell therapy (Kuriyan et al., 2017; Hinkle et al., 2021) and damage to retinal tissues as exosomes have low immunogenicity, toxicity and good biocompatibility and stability (Samanta et al., 2018).

Isolation and characterization criteria for exosomes

Exosomes can be isolated through different methods which each have their strengths and weaknesses (Thery et al., 2006). These include ultra-centrifugation, sucrose gradients, and polyethylene glycol-based centrifugation. Historically, the terms “exosome” and “extracellular vesicles” have been used interchangeably in the literature base (Mead and Witwer, 2018). However, since exosomes refer to EVs formed by a specific biogenesis pathway, the International Society for Extracellular Vesicles (ISEV) recommends that unless this biogenesis pathway is confirmed, they should instead be referred to as “small EVs” (Théry and Witwer, 2018). The papers reviewed in this manuscript do not follow these guidelines and typically just call them “exosomes”, and there is still much contention in the field over preferences for different nomenclature (Witwer and Théry, 2019). Thus, while we fully support the ISEV guidelines, we will continue to refer to them as exosomes in this manuscript to avoid confusion and maintain consistency with the studies being reviewed. We will however highlight any discrepancies or shortcomings in the isolation techniques of the reviewed papers. Typically, exosomes/EVs will have undergone ultracentrifugation and another isolation method of either ultrafiltration (such as 0.22 μm filter membrane or tangential flow filtration) or density gradient centrifugation (such as sucrose), which gives highly purified exosome solutions (Chen et al., 2021a). In addition, filtered exosomes should be sized 30-150 nm, have cup-shaped morphological characteristics, and have common markers such as CD63, CD9, and CD13.

Focus of this review article

Numerous studies have investigated the role of exosomes either as passive biomarkers in AMD (Bisutico et al., 2013; Kang et al., 2014), or potential mediators of the disease (Wang et al., 2014; Kienzlar-Aroca et al., 2016; Kannan et al., 2016; Atienza-Aroca et al., 2018; Elbay et al., 2019; Mukai et al., 2021) and this has been reviewed alongside their role in other eye diseases (Klingeborn et al., 2017). There is continued research on the use of exosomes as a drug delivery vehicle into the eye, utilizing exosome ability to deliver compounds while loading them with known therapeutic agents (Wassmer et al., 2017; Zhang et al., 2019a; Dong et al., 2021). These studies are too few however to provide a compelling review, as this area of research is still incredibly novel.

This review will focus on the therapeutic potential of exosomes, which, within a vibrant area of research, has slowly had several exciting studies published. Exosomal therapy may act as a potential cell-free therapy that aids rehabilitation and improvement of vision in AMD.

Search Strategy

The studies cited in this review were identified through PubMed and Google Scholar, alongside any in-text citations found within this primary body of literature. Keywords used are the same as the keywords associated with this manuscript.

Exosomal Therapy in Age-Related Macular Degeneration Models

Below we will discuss the therapeutic effects of exosomes in different models of AMD, with a brief introduction to the model used. These studies have had their findings summarized in Additional Table 1.
MNU mouse/rat model

Another model of AMD is the N-methyl-N-nitosourea (MNU) model, which suggests that the retina and choroid are involved in oxidative and lipofuscinogenesis. In the model, MNU, a DNA alkylating agent which is toxic to the retina, is injected intraperitoneally into rats, inducing retinal photoreceptor degeneration through photoreceptor outer segment loss, evidenced by the thinning of ONL (Chen et al., 2014).

Exosome/EV therapy in MNU mouse/rat model

Using the MNU model, exosomes derived from human adult retinal pigment epithelial cells labeled lipoproteins (BMSC-exos) (Wang et al., 2021), as well as exosomes derived from mouse bone marrow (BMSC-exos) (Deng et al., 2021) demonstrated common findings with Yu et al. (2016). Both papers found that exosome treatment significantly preserved ONL thickness and scotopic ERG amplitudes of alpha and beta waves, retained normal retinal architecture and protected cone photoreceptors as shown in immunohistochemical staining (Wang et al., 2021) and TUNEL analysis (Deng et al., 2021).

Furthermore, Wang et al. (2021) also showed copious punctate and opsin staining in the retina which indicated cone photoreceptors were undamaged, with M & S cones best protected against MNU-induced injury, whereas no staining was found in the untreated MNU control group. Visual acuity, contrast sensitivity, and optokinetic behaviors tests displayed all-round protection of sensitivity, and optokinetic behaviors tests displayed all-round protection of visual function. The authors explained that the mechanism of action was through the inhibition of MCP-1 mRNA expression, as evidenced by the reversal of the alleviated effects when injected with exogenous MCP-1.

Discussion

Concluding based on the limited articles available on retinal exosomal therapy, there is congruent evidence that exosomes or EVs from respective origins specified in their papers can preserve the retinal structure and layers, protect photoreceptors from apoptosis as well as retinal functional and ERG response (Yu et al., 2016; He et al., 2018; Bian et al., 2020; Deng et al., 2021; Chen et al., 2021b; Wang et al., 2021) in multiple models of AMD. In all studies except Park et al. (2021), exosomes were also observed to exert a therapeutic effect through influencing the expression of various proteins, cytokines, growth factors and mRNA levels. Few papers have also highlighted exosomes’ role in blood vessel leakage suppression and CNV inhibition (Hajrasoulia et al., 2013; He et al., 2012). While there is limited evidence performed on a different target disease, the exact mechanism of action has yet to be fully determined. Some papers have also found rapid diffusion of exosomes or EVs upon administration (Hajrasoulia et al., 2013, Yu et al., 2016) and long-lasting therapeutic effects up to 21 days (Bian et al., 2020; Deng et al., 2021), showing optimistic prospects of exosomes as a possible alternative or adjunctive treatment to anti-VEGF injections with less risk.

Differential findings were observed by Park et al. (2021) on BMSC-exos and Hajrasoulia et al. (2013) on ARPE-exos in comparison to other literature. A lack of therapeutic effect in the RCS model may be due to methodology discrepancy, as BMSCs were cultured under 1% oxidative stress for 48 hours before exosome isolation and characterization, which could have caused stress-induced changes in the exosomal cargo. Conflicting results found between ARPE-exos and RPE-exos on exosome therapeutic effects may be due to a difference in expressed complement factors between mouse and humans RPE exosomes (Bennis et al., 2015), or a potential difference in protein composition between normal RPE and ARPE-19 cell line. Another reason for the lack of therapeutic effect from RPE-exos may be due to the injection of RPE cells (and potentially their exosomes) in the pathogenesis of AMD, including drusen formation, involvement with macrophages and stimulation of inflammatory cytokines, angiogenesis and oxidative stress formation (Wang et al., 2009; Atienzar-Aroca et al., 2016, 2018; Mukai et al., 2021; Wang et al., 2018). It was speculated that exosomes target photoreceptors directly to exert their therapeutic effects, as BMSC-exos labeled with PKH26 dye aggregated linearly in vivo (Deng et al., 2021). While there is valid evidence of exosome or EVs as a potential therapy, there is congruent evidence that exosomes or EVs from respective origins specified in their papers can preserve the retinal structure and layers, protect photoreceptors from apoptosis as well as retinal functional and ERG response (Yu et al., 2016; He et al., 2018; Bian et al., 2020; Deng et al., 2021; Chen et al., 2021b; Wang et al., 2021) in multiple models of AMD. In all studies except Park et al. (2021), exosomes were also observed to exert a therapeutic effect through influencing the expression of various proteins, cytokines, growth factors and mRNA levels. Few papers have also highlighted exosomes’ role in blood vessel leakage suppression and CNV inhibition (Hajrasoulia et al., 2013; He et al., 2012). While there is limited evidence performed on a different target disease, the exact mechanism of action has yet to be fully determined. Some papers have also found rapid diffusion of exosomes or EVs upon administration (Hajrasoulia et al., 2013, Yu et al., 2016) and long-lasting therapeutic effects up to 21 days (Bian et al., 2020; Deng et al., 2021), showing optimistic prospects of exosomes as a possible alternative or adjunctive treatment to anti-VEGF injections with less risk.

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disrupted compared to mouse adipose- or hucMSC-exas in the MNV model, again, despite a lower dose used (Yu et al., 2016; Deng et al., 2021). These findings demonstrate a strong need for direct research into the relationship between exosomal therapy and oxidative stress. The consistency and broadness also support that exosomal properties depend largely on which cell in the body it is released from and as a result can alter the function and efficacy of exosomes.

In the current literature, exosome therapy is tested on rodent models including MNV, laser retinal injury, RCS, and blue light-induced oxidative stress. While these models partially cover the oxidative stress, local inflammation, and neovascularization of AMD’s pathophysiology, there are currently no in vivo rodent models available on drusen formation and subsequent buildup of photoreceptor waste product. In addition, current AMD animal models are far from perfect, mimicking only limited parts of AMD’s multifactorial nature and they do not reflect the exact pathway undergone and pathological features such as the chorioretinal environment in AMD (Shah et al., 2015). Furthermore, current models including MNV and laser retinal injury induce rapid and indiscriminate damage to the retina rather than a progressive development like in AMD, demonstrating a need for further model development or uses of other animal species.

Exosome therapy in other retinal diseases

Exosomes have also been researched substantially as a therapeutic tool in various other retinal diseases including diabetic retinopathy (Safwat et al., 2018; Li et al., 2021), glaucoma (Mead et al., 2018a, b) and retinopathy of prematurity (Xu et al., 2019; Cai et al., 2021). In addition, there are also various ongoing clinical trials including on dry eye and diabetes that show great promise in regenerative cell-free therapy and that exosome treatment is not far from reach.

Future Research Directions

Exosomes are an incredibly novel area of research, particularly with regard to their use as a therapy. This means a variety of hurdles and unknowns still exist in the quest to translate these compounds into viable therapies for AMD patients.

Definition, quantification, isolation, and characterization

The exact definition of exosomes and the quantification criteria has yet to reach a consensus (Witwer and Théry, 2019) and while the guidelines established by ISAVE go some way to rectify this, it is still contentious in the field and, as evidenced by the studies discussed here, largely ignored. In addition, various methods for the isolation and characterization of exosomes are available, all of which affect the purity and heterogeneity of the sample. Therefore, while highly purified exosomes have been isolated from many different bodily fluids, there is difficulty in comparing study outcomes with similar studies using different isolation/purification protocols. More research should be invested into methods of purifying exosomes to give a good yield of pure exosomes with a balance over cost as well as head-to-head comparisons between methods.

Mass scale production

In addition to overcoming isolation and characterization challenges through further research, to facilitate treatment across the world, large-scale production of exosomes is necessary. Isolation methods to produce a high yield to match the demand without compromising cell behavior are needed and demand further investigation (Whitford and Gutersam, 2019).

Method of administration

There is a huge variety of administration methods available. Further investigation on form of administration, including periocular, subretinal, and intravitreal injections (Del Amo et al., 2017) may also prove useful to compare the efficacy and duration of effect as well as assess risks for injection. The delivery of exosomes into the systemic circulation to treat the eye is also a potential given their ability to cross the blood-retinal barrier but, again, needs further investigation regarding efficacy and toxicity.

Exosome cargo

As exosomes were first discovered 30 years ago, there are still complex aspects of exosomes within their cargo that have yet to be fully understood, especially due to their heterogeneity and different expression across different cells. Consistent concentrations, cargo contents, and stage of differentiation are required to facilitate exosome therapy, hence further research should be allocated to identifying and comparing cargo contents and differentiation stage across the same cell type. Despite these low number of studies, however, is a growing comprehensive database of exosomal cargo (http://www.exocarta.org/) that include many cargo analyses performed on exosomes.

Toxicology

If exosomal therapy is translational, exosomes will eventually be administered into humans. While there have been toxicology studies of exosomes on animals (Zhu et al., 2017) demonstrating their safety, some studies have highlighted the potential for toxicity (Cho et al., 2018) that correlates with the formation of exosomal aggregates (Rothe et al., 2019). The limited studies however highlight that toxicity of exosomes in specific tissues is limited, and with respect to AMD, these potential toxic effects in the eye should be investigated contemporaneously with any development of exosome-based therapies.

Conclusion

Exosomes or EVs harbor immense potential as viable therapy both in retinal disease and age-related macular degeneration. However, further research is required to solidify findings as well as investigate the mechanism of action.

Author contributions: LLCC: Conception and design of the manuscript, collection and/or assembly of data, data analysis and interpretation, and manuscript writing; BTM: Conception and design and manuscript writing. Both authors contributed equally to this article.

Conflicts of interest: The authors declare no conflicts of interest.

Availability of data and materials: All data generated or analyzed during this study are included in this published article and its supplementary information files.

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Open peer reviewer: Amany Tawfik, Oakland University, USA.

Additional files: Additional file 1: Open peer review report 1.

Additional Table 1: Summary of exosomal or extracellular vesicle therapeutic effect on AMD model.

References


