• Hypothesis •

Magnetic nanoparticles conjugated with "RPE cell -MCP-1 antibody -VEGF antibody" compounds for the targeted therapy of age-related macular degeneration: a hypothesis

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Abstract

• Age-related macular degeneration (AMD) is the leading cause of vision loss in the elderly throughout the world. Treatment of AMD utilizing retinal pigment epithelium (RPE) transplantation represents a promising therapy. However, simplex RPE transplantation can only replace the diseased RPE cells, but has no abilities to stop the development of AMD. It has been indicated that oxidization triggers the development of AMD by inducing the dysfunction and degeneration of RPE cells, which results in the upregulation of local monocyte chemotactic protein-1 (MCP-1) expression. MCP-1 induces macrophage recruiment which triggers local inflammation. As a result, the expression of vascular endothelial growth factor (VEGF) is upregulated by MCP-1 mediated inflammation and results in the formation of choroidal neovascularization (CNV). We accordingly propose a targeted therapy of AMD by subretinal transplanting the compound of RPE cell, MCP-1 antibody, and VEGF antibody and using a magnetic system to guide RPE cell compounds conjugated with superparamagnetic iron oxide nanoparticles (SPIONs). Furthermore, SPION-labelled RPE cells can be tracked and detected *in vivo* by non-invasive magnetic resonance imaging (MRI). This novel RPE cell transplantation methodology seems very promising to provide a new therapeutic approach for the treatment of AMD.

• KEYWORDS: age-related macular degeneration; retinal pigment epithelium; superparamagnetic iron oxide nanoparticles; RPE cell transplantation; targeted therapy

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INTRODUCTION

Age-related macular degeneration (AMD) is a multifactorial disease that represents the most common cause of irreversible visual impairment among people over the age of 50 throughout the world[1-2]. RPE cell defect has been implicated in and perhaps primary to some pathological changes of the retina in AMD cases[3]. Clinically, the disease is classified into slowly progressing atrophic-dry AMD (in majority of cases) with advanced geographic atrophy, and rapidly progressing neovascular (exudative)-wet AMD (10%-15% of all cases) with choroidal neovascularization (CNV)[4]. Till now, there is no proved therapy for dry AMD. Despite new medical and surgical interventions for wet AMD, none of up-to-now used treatments can definitely cure the disease or reverse its course.

The occurrence of drusen between the retinal pigment epithelium (RPE) layer and Bruch membrane and the formation of CNV are the two typical pathological features of AMD. Vision damage of AMD patients mainly results from the progressive atrophy of RPE layer induced by drusen and the retinal hemorrhage, macular edma, and histological destruction due to CNV. Current available therapies, such as photodynamic therapy, anti-vascular endothelial growth factor (VEGF) therapy, submacular surgeries, are mainly focused on eliminating CNV[5-7]. Although these methods can partially improve the vision of AMD patients by reducing CNV lesion and alleviating retinal edma or hemorrhage, repetitive treatments are requested. Furthermore, in most cases, the progress of vision loss can not be stopped by using these therapies. The limited benefits of current medical and surgical interventions for AMD accentuate the need to establish more effective therapies. Currently, RPE transplantation is considered to have the potential to rescue vision in patients with AMD, because the primary lesion in this disease occurs in the RPE layer[8]. Howerver, mere RPE transplantation can not stop the progess of AMD on account of multiple factors that cause this disease.

Oxidization is believed to trigger the development of AMD by inducing the dysfunction and degeneration of RPE cells, which results in the upregulation of RPE cell derivedmonocyte chemotactic protein-1 (MCP-1) expression[9-12]. MCP-1 induces macrophage recruiment which initiates local inflammation. Subsequently, VEGF secreted by accumulated macrophages is upregulated by MCP-1 mediated inflammation and results in the formation of CNV which seriously damages eyesight[13-14]. Now the problem is coming: is it possible to stop the three pivotal pathological progresses of AMD at the same time? In this article, a novel therapy is proposed that triple transplantation of "RPE cell -MCP-1 antibody -VEGF antibody" compounds maybe a promising approach to prevent AMD development and CNV formation once and for all.

WHAT IS ALREADY KNOWN?

The previous researches have demonstrated the possible pathological progress of AMD as follows. 1) Oxidization is the primary risk factor of AMD development, which initiates the pathological process of AMD. 2) Oxidization actuates the dysfuntion and degeneration of RPE cells, which results in the upregulation of local MCP-1 expression. To restore the structure and function of RPE layer is the primary task for AMD treatments. 3) MCP-1 activates macrophage recruiment which stimulates local inflammation and leads to the increased expression of VEGF in retina. To supress MCP-1 chemotatic activities is a necessary step to prevent the development of AMD. 4) Increased VEGF facilitates the formation of CNV which seriously damages eyesight. To inhibit VEGF angiogenic funtion is very crucial to stop CNV lesion. 5) RPE cell transplantation has emerged as a novel therapeutic option for AMD. 6) Superparamagnetic iron oxide nanoparticles (SPIONs) have successfully labeled different mammalian cell types and are one of the prefered methods for cell labeling and tracking in preclinical and clinical studies. SPIONs can be further functioned by surface coating using proteins, peptides, antibodies, ploymers, DNA, and so on.

Oxidization is an inescapable factors in human daily life. Therefore, AMD treatments should be focused on how to stop oxidization induced pathological progresses including RPE cell degeneration, MCP-1 mediated local inflammation, and VEGF derived CNV formation rather than oxidizaiton itself.

RPE transplantation has been employed to reestablish the monolayer structure of RPE cells and restore central vision in AMD cases[8]. Nevertheless, mere RPE transplantation can not suppress MCP-1 induced local inflammation and VEGF derived CNV formation. We speculate that MCP-1 and VEGF antibodies modified RPE cell transplantation may be a promising strategy to resolve these problems once and for all. SPIONs are seem to be an ideal approach for RPE cell labeling and tracking.

THE HYPOTHESIS

We propose that transplanting SPIONs-interlinked "RPE cells-MCP-1 antibody - VEGF antibody" compounds maybe a praticable way to prevent the development of AMD at the very early stage. The three-in-one combination is able to reestablish RPE layer by RPE cell replenishment, inhibit local inflammation by reducing MCP-1 chemotactic activities, and prevent CNV formation by attenuating VEGF angiogenic functions either in sequence or simultaneously. Furthermore, the three-in-one compound can be prisely localized to the macular site *via* magnetic navigation and be non-invasively detected by magnetic resonance imaging (MRI) *in vivo*.

DISCUSSION

AMD is a major cause of blindness in the elderly worldwide[1-2]. Phenotypically, AMD can be divided into two main forms: dry (atrophic) and wet (exudative) types and further subdivided into early and late-stage diseases. At present, cure for AMD and especially effecitive interventions in its early stage is lacking due to its multi-pathogenic factors including RPE cell dysfunction, MCP-1 mediated local inflammation, and VEGF derived CNV formation. RPE cell transplantation is currently considered to be an excellent strategy for the treatment of AMD and demonstrates improvement in visual function in AMD cases[8]. Nevertheless, the therapeutic effect eventually lost, most likely due to inefficient interruption of all risk fators for AMD. In this article, an upgraded RPE transplantation conjugated with MCP-1 and VEGF antibodies is proposed to be a promising therapy for AMD once and for all.

Transplantation of "RPE cell -MCP-1 antibody -VEGF antibody" compounds is possibly much more effective in preventing AMD development than mere RPE cell transplantation, because the three-in-one combination may have the potential to reestablish RPE layer by RPE cell replenishment, inhibit local inflammation by reducing MCP-1 chemotactic activities, and prevent CNV formation by attenuating VEGF angiogenic functions either in sequence or simultaneously. However, it is a big challenge to constitute "RPE cells - MCP-1 antibody -VEGF antibody" compounds. SPIONs are the hopeful candidate to make our hypothesis come true. Recent studies have demonstrate that SPIONs represents a generalizable platform technology for regenerative medicine. SPIONs are reported to cure acute myocardial infarction by conjugating with two types of antibodies (one against antigens on therapeutic cells and the other directed at injured cells) which link the therapeutic cells to the injured cells[15]. A previous study has reported that it is feasible to construct and deliver RPE cell sheets *in vitro*, using magnetite nanoparticles and magnetic force[16]. SPIONs are also capable of conjugating with multi-antibodies and various cells at the same time to produce magnetic multifunctional cell engager[15,17], which makes it possible to link VEGF and MCP-1 antibodies to the therapeutic RPE cells.

Meanwhile, current RPE cell transplantation is limited by inefficient delivery strategies of cells into the macular area and lack of non-invasive tracking methods. SPIONs have been used to label, localize and visualize various cell types

with MRI *in vivo* and *in vitro*, such as bone marrow-derived cells, dendritic cells, and mesenchymal cells[18-20]. Therefore, it is very possible that SPIONs can not only localize RPE cell compounds prisely to the subretinal space of the macular site, but also enable them to be non-invasively tracked and detected *in vivo*.

However, safety is a main concern, due to the unique and unsual properties acquired by compounds forming particles with SPIONs. Relatively low toxicity has been found even when SPIONs concentrations as high as 100 μg/mL were used[21], yet long-term effects in humans have not been reported. Several toxicity parameters, including a possible negative effect on the physiology of labeled RPE cells, promotion of a proinflammatory environment and cytotoxicity to the recipient retina, have to been assessed before clinical application.

In brief, our hypothesis can be summarized as follows: MCP-1 and VEGF antibodies are linked by SPIONs to RPE cells to produce all-in-one compounds which can not only be prisely targeted to renovate impaired RPE layer of the macular site *via* magnetic navigation, but also be non-invasively tracked and visualized by MRI *in vivo*. The novel approach will be a promising strategy for AMD treatments. However, the possible cytotoxicity of SPIONs in subretinal space to the surrounding tissues has to be considered ahead of time.

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