·Review·

Research progress of stem cells on glaucomatous optic nerve injury

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Abstract

• **Glaucoma, the second leading cause of blindness, is an irreversible optic neuropathy. The mechanism of optic nerve injury caused by glaucoma is undefined at present. There is no effective treatment method for the injury. Stem cells have the capacity of self –renewal and differentiation. These two features have made them become the research focus on improving the injury at present. This paper reviews the application progress on different types of stem cells therapy for optic nerve injury caused by glaucoma.**

• **KEYWORDS:** stem cells; glaucoma; optic nerve injury; research progress
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INTRODUCTION

Glaucoma, defined as optic neuropathy with distinctive injury of the optic nerve and defect of field vision by some scholars [1], will cause blindness without timely and proper treatment. Epidemiological studies point out that the number of people with glaucoma will reach 79 600 000 worldwide and 6 000 000 in China by 2020 [2]. It is the high probability of blindness that has made glaucoma become an important bottleneck in the prevention of blindness. Currently, drugs and surgical operation are the only ways to treat glaucoma by achieving the purpose of lowering intraocular pressure. But how to improve the injury of optic nerve and the defect of visual field has always been a problem puzzling ophthalmologists for a long time.

At the beginning of twenty-first century, researches on stem cells were elected as one of the ten most important research fields for the first time in twenty-first century [3]. Stem cells are a type of cells with the capability of self-renewal and differentiation. The pathological basis of optic nerve injury in glaucoma is degeneration and death of retinal ganglion cells (RGCs), which do not have the ability of regeneration. If injured, they will never be repaired. Fortunately, it is possible that the therapy of stem cells can replace the apoptosis and protect the damaged cells [4], therefore bring a new breakthrough for the treatment of glaucomatous optic nerve injury.

MECHANISM OF OPTIC NERVE INJURY IN GLAUCOMA

The mechanism of optic nerve injury is not clear. Almost all of the researchers believe that a combination of factors causes the injury. Quigley *et al* [5] show that high intraocular pressure can damage lamina cribrosa of sclera, thus it blocks the optic nerve axons transport and damages cellular metabolism. The abnormal blood flow is also considered as the cause of optic nerve injury. A study [6] has shown that hypertension can increase the risk of glaucoma. Because hypertension is a systemic disease with abnormal blood flow, it will not only impair the function of automatic regulation of ciliary posterior circulation, but also cause the vascular injury which can lead to reduced anterior optic nerve blood flow. All of those factors stimulate the damage to RGCs. Zhao *et al* [7] think that lots of glutamate in the retinal tissue will release in the condition of high intraocular pressure, then the transport corridor of intracellular Ca^{2+} will become abnormal, which will activate nitricoxide synthase (NOS), produce NO, and fracture nuclear DNA , thus cause the RGCs injury and apoptosis. Professor Ningli Wang has put forward a theory that the increase of pressure gradient between brain and eye causes the optic nerve injury at the World Glaucoma Conference (WGC) in 2015. The discovery answers the question why the optic nerve injury still occurs in glaucoma patients with normal intraocular pressure. In addition, there is

also evidence[8] showing that autoimmunity may lead to injury of optic nerve by direct autoantibody or indirectly imitating sensitizing antigen to induce autoimmune reaction, and the immunity also can regulate death of RGCs by monitoring immune system signaling pathway.

Application Progress on Different Types of Stem Cells in Optic Nerve Injury At present, the stem cells which are closest translation to the clinical are embryonic stem cells (ESCs), adult stem cells and induced pluripotent stem cells (iPSCs). ESCs derive from the inner cell mass of early embryos of mammalian. Adult stem cells exist in specific tissues, such as mesenchymal stem cells (MSCs), limbal stem cells and retinal stem cells (RSCs), *etc*. iPSCs can be induced from differentiated somatic cells.

Embryonic stem cells ESCs are kind of stem cells which have powerful ability of proliferation. Therefore they have the potential of differentiation into all types of cells in the body [9]. Hambright *et al* [10] have found that transplanting ESCs into the subretinal space of mice could differentiate them into photoreceptor cell. Moreover, through the outer retina, ESCs can be effectively integrated into the RGC layer and inner nuclear layer. Main way of application of ESCs is direct intraocular injection. In early times, ESCs were injected into the vitreous cavity and then moved to the corresponding target on the retina. However, not all of the virus will be transferred to the target position. This method just achieves limited therapeutic effect. With the therapy of stem cells replacement becoming more and more popular, Wert *et al* [11] have provided a video technology that allows human ESCs to be injected directly into the subretinal space of mice. By this technique, the stem cells can be ensured to be placed in an effective treatment position in ophthalmic diseases.

However, because the study on ESCs requires destruction of embryos, which are life forms existent life form in the uterus after the combination of male germ cells and female germ cells, the utilizing of ESCs has caused the ethical controversy. In the meantime, possible rejection after the implantation of ESCs also has made the study on ESCs face a dilemma. How to solve this problem needs further exploring.

Adult Stem Cells

Mesenchymal stem cells MSCs are existing in the connective tissue (bone marrow, fat, vascular stem cells, *etc*).

Bone marrow-mesenchymal stem cells Bone marrow-mesenchymal stem cells (BMSCs) have the ability of differentiation, regulating the response of repairing injury of host cell and promoting endogenous progress of repair [12]. BMSCs can be directly obtained from autologous bone marrow, thus it avoids immune rejection and ethical issues. Xu *et al* [13] find that BMSCs can be transplanted into subretinal space by the form of suspensions. After 15d BMSCs survive in the space and mainly distribute in the layer of retinal pigment epithelium (RPE), cone and rod cell. Recent research [14] shows that co-cultivation BMSCs and retinal pigment epithelial cells can differentiate into photoreceptor cells and retinal cells. Hu *et al* [15] transplant BMSCs for the treatment of glaucoma in rats modeled by laser induced high intraocular pressure and find that BMSCs can promote the survival of RGCs. Qu *et al* [16] find that the overexpression of Fas/FasL may lead to apoptosis of retinal target cells. The intravitreal injection of BMSCs can decrease the positive expression of Fas/FasL. Thus they speculate that inhibition of Fas/FasL overexpression and then inhibition of apoptosis may be one of the mechanisms of BMSCs. Jiang *et al* [17] transplant BMSCs into animal models of glaucoma and show that BMSCs can reduce the expression of glial fibrillary acidic protein (GFAP) and MMP-2 mRNA and have effect of reducing apoptosis of ganglion cells. The expression of GFAP is closely related with the severity of retinal injury. MMP-2 is involved in the process of normal physiology and damage of extracellular matrix of many diseases, such as wound healing, tissue remodeling, inflammatory reaction, apoptosis of cells, differentiation and proliferation of cells, *etc* [18]. MMP-2 is rarely detected in normal eyes, while in pathological conditions, the expression level will increase if MMP-2 is affected by some factors such as stimulation, tissue damage and inflammation [19-20]. At the same time, in their further study, Jiang *et al* [17] find that BMSCs play a role in protection and repair of nerve mainly by regulating the intraocular microenvironment. BMSCs provide a stable micro environment to reduce or delay the formation of scar tissue and help the reconstruction of injury. They also think that because intravenous injection is a simple procedure without eye injury, allowing for the flexible control of the dose of cells, which is more practical than the local injection, and therefore it is a promising method for the treatment of retinal injury. He *et al* [21] think that ribosomal protein L13a (RPL13A), and the combination of Cyclin A (CYCA) and peptidylprolyl isomerase A (PPIA) are identified as the most suitable reference genes for RT-qPCR in neuronal cells differentiated from BMSCs.

Adipose-derived Stem Cells Because adipose tissue and mveloid tissue both belong to mesodermal tissue, adipose tissue should also contain similar stem cells. Haddad-Mashadrizeh *et al* [22] transplant human adipose-derived stem cells (ASCs) into the rat eye and find that ASCs can survive for 90d in vitreous, and 6mo or longer in the other tissue of eyes. Several scholars' [23-24] researches confirm that ASCs have protective effect on retinal injury. Stromalvascular component of adipose tissue contain MSCs, suggesting that ASCs may play a role by secreting neurotrophic factors [23]. Xu[25] found that stem cells derived from human orbit adipose tissue were potential directional differentiate into the cells similar to retinal photoreceptor cells. Compared with mveloid

tissue, adipose tissue is easier to be obtained, simpler to culture, and more suitable for large-scale application.

Retinal Stem Cells Zheng [26] identified that fetal rats RSCs can express retinal nerve cells and glial cells after separating, culturing, and inducing differentiation. A number of different cellular sources of RSCs have been identified in the vertebrate retina. These include RSCs at the retinal margin, pigmented cells in the ciliary body, iris, RPE, and Müller cells within the retina [27]. Jasty *et al* [28] think that the iris and ciliary pigment epithelial cells can generate neuropheres containing progenitor cells in the presence of mitogens and are capable of producing different types of retinal cell demonstrated by RT-PCR and immunocytochemistry. Tropepe V [29], Canadian scientist, has found that RSCs exist in the RPE at the edge region of ciliary body in adult mice. In the vertebrate retina, Müller cells are the main support cell [30]. Growth factors, transcription factors, and extracellular matrix regulate differentiation and proliferation through some important signaling pathways, such as MAPK, Notch and Wnt [31-34], thus these factors greatly promote the capability of dedifferentiation and regeneration. However, it is very difficult to activate the proliferation and dedifferentiation in mammals after retinal injury and the number of differentiated Müller cells are quietly limited. How to control the Müller cells to differentiate into neurons to repair injury retina is the focus and main difficulty in current research.

Because the RSCs can directly differentiate into retinal nerve cells *in vivo* micro environment, and other types of stem cells may turn into cells just like retinal nerve cells by inducing differentiation, RSCs is the most direct and effective treatment for retinal disease.

Induced Pluripotent Stem Cells IPSCs are induced by means of reprogramming differentiated functional somatic cells, which are similar to ESCs in terms of high proliferation, proficiency of multilineage differentiation and having their own genetic material [35]. IPSCs can be obtained from blood cells, vesicle stromal cells and lymphocyte [36-38]. Zhou *et al* 's [39] research show that iPSCs can differentiate into photoreceptor cells and these cells can integrate into the injury optic nerve in pigs. Tucker *et al* [40] make mouse epidermal fibroblasts induced into iPSCs and differentiate into photoreceptor precursor cells. Then the retinal progenitor cells, photoreceptor precursor cells, and mature photoreceptor cells are formatted after 33d. They form a new synapse integrating into the retina with the host cells. Wang *et al* 's [41] culture system provides a new method, that is a retinal differentiation medium (RDM) without the use of small molecular compounds, for generating human PSC-derived retinal cells efficiently differentiated into retinal cells. They suggest that the culture system, for the first time, might be used in human transplantation. However, there are also many problems existing in iPSCs: for example, the mechanism of iPSCs differentiating into retinal cells is not fully elucidated and the efficiency of differentiating into purpose cells is still low.

LIMITATIONS AND PROSPECTS

Therapy of stem cells can improve the effect of clinical treatment on glaucoma and visual acuity. There are broad prospects for stem cells in the treatment of optic nerve injury and can bring admirable social and economic benefits. But stem cells also face a number of peculiarly tricky problems: 1) stem cells have the ability of multi-directional differentiation, and, of course, they will have the potential for pathological changes, but the research on issues of security is still lacking at present; 2) the efficiency of stem cells differentiating into specific cells and the rate of survival in the retina are still low; 3) the mechanism of the occurrence and development of glaucoma is ambiguous yet and the time of clinical application of stem cells is relatively short, so the long-term effects of stem cells implanting into eyes are still unknown. These problems need to be further explored before we can do more to promote the advantages of stem cells in clinical applications and bring light to more patients with glaucoma.

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