Stem cells therapy research for the treatment of parasitic infections

Review Article

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ABSTRACT

Parasitic diseases have significant global economic, environmental, and public health impacts. In recent years, stem cells therapy has become a very promising and advanced scientific research topic. Since stem cells are the primary, unspecialized mother of all cells, they have the ability to differentiate into specialized cells. Besides, they have a remarkable potential to develop into many different cell types in the body to replace the damaged tissues. Recently, researchers experimentally investigated the application of these cells to treat parasitic diseases such as schistosomiasis, malaria, trypanosomiasis, leishmaniasis and toxoplasmosis with improvement of the function of the involved tissue and organs. This review summarized the up-to-date application of stem cell technology for treatment and/or protection against parasitic diseases. We aimed to highlight how these approaches affected the parasite–host interactions and contribute to the identification of novel targets for therapies and vaccines.

Keywords: malaria; MSCs; protection; schistosomiasis; stem cells; therapy; toxoplasmosis.

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INTRODUCTION

Stem cell therapy is an interventional treatment that introduces new cells into damaged tissues to help in treating many diseases and injuries. They are the body's original cells from which other cells with specialized functions are generated. Stem cells are capable of constructing all the body tissues including their differentiation properties and continuous self-renewal. Several stem cells offer great potential uses for therapeutic purposes in tissues repair and regeneration[1]. Takahashi and Yamanaka[2] discovered that it was possible to reprogram multipotent stem cells (MPSCs) to the pluripotent stem cells (PSCs) state. Notably, MPSCs are capable of self-renewing into any specialized cell type in any organ, and are able to give rise to multiple cells within a lineage. Whereas PSCs are embryonic stem cells capable of differentiation into all primary germ cells layers, excluding the extraembryonic placental cells, i.e., giving rise to all embryonic and adult lineages. Signals that affect the stem cell specialization process can be divided into external, physical or chemical secretions by the surrounding tissue; and internal signals under gene control. Stem cells also act as internal repair systems of the body with unlimited production of new cells. Besides, stem cell activity depends on the organ in which they reside, as in the bone marrow (BM) where their division is constant. However, in organs such as the pancreas, division only occurs under special physiological conditions[2].

Oligopotent stem cells (OSCs) can differentiate into several cell types but with a limit as these cells are able to self-renew within specific tissues like lymphoid or myeloid stem cells. Unipotent stem cells (USCs) are characterized by the narrowest differentiation capabilities. These cells are only able to form one cell type, e.g., dermatocytes, but have a special property of dividing repeatedly. This feature renders them a promising candidate for therapeutic use in regenerative medicine[3]. The mesenchymal stem cells (MSCs) are another first generation stem cell type of multipotent cells that can be isolated from different tissues, including BM, adipose tissue, umbilical cord and others[4]. As early as 2008, Uccelli[5] showed that MSCs can interact with cells of both innate and adaptive immune responses, leading to immunomodulation that included anti-proliferative and anti-inflammatory effects, mainly as a result of their production of high levels of interferon-gamma (IFN-γ) and tumor necrosis factor-α (TNF-α)[5].

Consequently, results of the cloning experiment became a great discovery since it was previously believed that cell differentiation was one-way only, but it was demonstrated that it was possible for a somatic cell to again acquire pluripotency[6]. Later, it was published that retrovirus-mediated transduction of mouse fibroblasts with four transcription factors (Oct-3/4, Sox2, KLF4, and c-Myc), that are expressed in embryonic stem cells, could affect fibroblasts to become pluripotent[7]. The Nobel prize in Physiology or Medicine in the year 2012 was awarded for the discovery that mature cells could be reprogrammed to become pluripotent[8].

The European Medicines Agency (EMA) in addition to the Food and Drug Administration (FDA) set several

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guidelines for the appropriate and safe use of stem cells transplantation as products of regenerative medicine requires approval from FDA before it is marketed for consumers. One of these guidelines is concerned with whether the cells are recovered from own body or another person's body. Moreover, stem cells obtained from cord blood for unrelated allogeneic use, are regulated by a FDA license form[9].

Considering the differentiation, division and origin of stem cells, Zakrzewski *et al*.[10] claimed in their review that totipotent stem cells (TSCs) which are cells that have the capacity to self-renew by dividing and developing into primary germ cell layers of embryo nicand extra-embryonic tissues such as placenta. Besides, totipotency has the greatest differentiation potential and allows stem cells to form both embryonic and extra-embryonic structures. One example of a totipotent cell is the zygote, formed after a sperm fertilizes an ovum[10]. The reviewers added that embryonic stem cells (ESCs) are derived from the inner cell mass of pre-implantation embryos. Another example are the induced pluripotent stem cells (iPSCs), which are derived from the epiblast layer of the implanted embryos. These iPSCs are artificially generated from the somatic cells, and their function is similar to PSCs. Their ease of culturing and utilization constitutes a very promising potential for regenerative medicine[10].

Until the year 2009, stem cells therapies research for parasitic diseases were still in their experimental stages[11]. A decade later, Matthews and Noulin[12] commented that stem cells treatment could not directly target the parasite itself, but could act through helping the patient fight and recover the post-infection. Therefore, the present review aims to focus on and discuss role of stem cells as advanced therapy in many parasitic diseases that will become a real therapeutic option for parasitic infections in the future.

Ongoing research in stem cells therapies for parasitic diseases

The therapeutic potentials of the stem cells for parasitic diseases such as Chagas'[13], malaria[14] and schistosomiasis[15] were introduced.

Schistosomiasis

Schistosomiasis is the second most prevalent parasitic disease in the world, causing clinical sequels. It is characterized mainly by granulomatous reactions around their eggs trapped in the tissues. The major pathologic lesions are the hepatic granuloma formation followed by hepatic fibrosis in advanced stages[15]. Another recent study supports that subsets of larvally derived stem cells are likely sources of adult stem cells and the germline. A novel identified gene serves as the earliest marker for the schistosome germline, which emerges inside the mammalian host and is responsible for disease pathology. This revealed that stem cell heterogeneity drives the propagation of the schistosome life cycle[16].

The role of schistosome germline attained the attention of many researchers. However, the key-player that regulates the outcome of germline stem cells (GSCs) reproduction into daughter cells or gametes was not fully understood. Single-cell RNA sequencing was conducted to discover the stem cells of the juvenile schistosomes. The sequencing revealed nine populations of stem cells divided into two basic categories: tissue-specific progenitors of epidermal, muscle, intestinal/parenchymal and neural lineages, and multipotent populations including GSCs. Transcriptomic categorization of the schistosome juvenile' GSCs revealed a conserved regulatory gene set that resided in the *Schistosoma* male germline. The regulatory gene contained a transcription factor (onecut) and a mRNA binding protein (boule) in its core. The expression of two components constituted a specific genetic program that controls the over-proliferation of schistosome GSCs and stimulates germ cell differentiation[16].

El-Shennawy *et al*.[17] attempted treatment with BM-MSCs in experimental schistosomiasis and reported significant reduction of granuloma size and the expression of alpha-smooth muscle actin (a fibrosis factor) in the hepatic cells. Considering the combined properties of MSCs, the investigators hypothesized that use of BM-MSCs as an adjuvant therapy would reduce the granulomatous response to *S. mansoni* eggs and enhance faster patient recovery. In an earlier trial on schistosomiasis, $CD133^{+}$ human umbilical cord blood stem cells were used to trigger the production of new blood vessels and promote vascularization, allowing better survival of the damaged cells[18]. In this report it was also suggested that adipose tissue stromal cells (ASC) therapy has immunomodulatory effects in schistosomiasis. These findings motivated researchers to use MSCs in an experimental therapeutic approach to minimize the hepatic lesions caused by schistosomiasis *mansoni*. Additionally the MSCs were used, in combination with praziquantel, to treat *S. japonicum* infection in a mouse model and the treatment resulted in smaller hepatic granulomas with little tissue damage[19]. Later, Miranda *et al*.[20] confirmed that also *S. mansoni* infected-mice treated with combined praziquantel and ASC had smaller granulomas and less tissue damage[20].

Stem cells derived from schistosome larvae were identified as a source of schistosome adult germline and stem cells. A unique gene that acts as a marker for the schistosome germline was isolated from the mammalian host and it was implicated for the schistosomiasis pathology[21]. Moreover, a recent study revealed that both schistosome adults and larvae stem

cells were involved in the regulation of life cycle stages development whether inside the snails or mammalian hosts. The sporocysts germinal cells were found to be involved in the asexual reproduction in the snail in addition to the neoblasts of adult worms which were involved in sexual reproduction within the mammalian hosts[22].

Malaria

Malaria is still considered a global problem for half of the world population[23]. Cerebral malaria is one of the most severe complications of infection with *P. falciparum* parasites, associated with neurological disorders. In addition to the development of anti-malarial drug-resistance, there is no effective and safe treatment available for cerebral malaria, notably for use in children who represent the majority of cases. Even patients who received standard antimalarial therapy at an early phase, are still at a high risk of mortality despite clearance of the parasite. Moreover, about 25% of the cerebral malaria survivors might develop neurological complications and cognitive impairment later in their life[24]. The MSCs were documented to play an important role in malarial infection including the accumulated cells in lymphoid organs during the progression of the malaria disease. During malaria infection, those MSCs were able to restore the functions of $CD4^+$, $CD8^+$ T cells and $CD34^+$ haematopoietic cells as well. Stem cell-based therapy for malaria could serve as a useful tool for attaining parasitic clearance and prevention of disease reactivation[25].

Souza *et al*.[14] reported that experimental treatment with BM-MSCs improved clearance of parasitized erythrocytes, increased regeneration of hepatocytes and Kupffer cells, increased numbers of astrocytes and oligodendrocytes in the brain and increased mice survival. Later, Thakur *et al*.[26] reported that because MSCs are the responsible cells for haematopoiesis reprograming in the bone marrow and lymphoid organs, the latter are considered the main organs that provide host protection against malaria.

During the first experimental use of stem cells therapy for malaria, it was found that hemopoietic MPSCs contributed to the host defense against *P. berghei* infection and increased the survival of infected mice[27]. Subsequently, transplantation of myeloid cells (produced by interleukin 7 receptor-alpha (IL-7Rα and c-Kithi progenitors), which were isolated from mice experimentally infected with *P. chabaudi*, was found to result in clearance of infected erythrocytes in the infected mice[27]. Later, the administration of MSCs against malaria in a mouse model of *P. berghei* infection was found to confer host resistance against malaria through increasing IL-12 production and suppressing IL-10 and regulatory T cell production[26]. This stem therapy reduced both parasitaemia and malaria pigment deposition in the spleen, liver, kidney and lung in an experimental mouse model of cerebral malaria. It was suggested that these findings might provide a new therapeutic option aginst cerebral malaria[28].

Chagas disease

The most successful attempt in application of stem cells therapy in parasitic diseases was recorded in Chagas' disease[13], caused by the parasite *T. cruzi*, and complicated with fatal heart diseases[29], in endemic areas of Latin America. Soares *et al.*[30] had reported that transplantation of BM mononuclear cells in a mouse model of Chagas disease proved to be effective in reducing myocarditis and fibrosis within 6 months after transplantation. In the same year a clinical trial conducted on a 52-year-old patient suffering from heart failure due to Chagas disease. Purified mononuclear cells, isolated from healthy BM were injected into the patient. A notable improved ventricular function was observed 30 days post-transplantation[31]. Hence, cardiac transplantation and BM stem cells therapy for Chagas disease patients received increasing research attention[32]. It was shown that repeated injections of granulocyte colony-stimulating factor (G-CSF), that mobilizes stem cells from the bone marrow, decreases inflammation and fibrosis in the hearts of chagasic mice[33]. A conducted study stated that MSC may constitute an interesting cell population to test in CCC. In the mouse model these cells proved to be effective but in human it is still in trial[34].

Leishmaniasis

Leishmaniasis is endemic in 98 countries throughout the world. It is transmitted by the bite of infected female phlebotomine sand flies, with more than 20 morphologically indistinguishable *Leishmania* spp. Visceral and cutaneous leishmaniasis were estimated to affect more than 350 million people, with an incidence varying between 1 and 1.5 million people[35,36]. Treatment options for this disease are limited and the available drugs are highly toxic. Moreover, domestic dogs being the main reservoir of the parasites supports the urgent need for effective therapy[37,38]. In order to demonstrate that stem cells could be infected with the main *Leishmania* spp. i.e., *L. major, L. tropica, L. donovani,* and *L. infantum*, a study using adipose tissue-derived mesenchymal stem cells (AD-MSCs) showed that stem cells could be a useful model to study treatment of leishmaniasis[39]. Attempted treatment with MSCs showed a significant regression in the cutaneous lesions produced by *L. major*[40]. Moreover, treatment with AD-MSCs combined with meglumine antimoniate reduced the cutaneous lesion size produced by *L. amazonensis*[41]. Additional combination of AD-MSCs with glucantime improved the cutaneous lesions' healing and decreased parasite burden caused by *L. major*[42].

Toxoplasmosis

The parasite responsible for toxoplasmosis is the apicomplexan *T. gondii* that is widely distributed throughout the world. It was estimated that about

30–50% of the world population are infected with this parasite[43]. Infection occurs *via* consumption of under-cooked meat of infected animals carrying the *Toxoplasma* tissue-cysts, in addition to food or water contaminated with oocysts shed by infected cats[44]. Etewa *et al*.[45] attempted Experimental treatment with BM-MSCs combined with spiramycin, pyrimethamine and folinic acid (SPF), and showed a significant decrease in the number and size of mice brain tissue cysts. When compared with a group tested by SCs as mono-therapy, a poor curable role was demonstrated in the livers, spleens, eyes and brain tissues studies. The same results were confirmed by the recruitment of CD8+ demonstrated in immunohistochemical sections of brain and spleen. Accordingly the investigators confirmed that BM MSCs alone have a poor therapeutic role than when combined with SPF for treatment of toxoplasmosis[45].

Hydatidosis

Alveolar and cystic echinococcosis are the larval stages of the cestode worms *E. multilocularis* and *E. granulosus*, respectively. Human intermediate hosts, acquire the infection by accidental oral ingestion of the tapeworms' eggs in stools of canine definitive hosts. Consequent development is by the unlimited asexual multiplication into metacestodes within host organs[46]. Besides surgical intervention to remove accessible cysts, drug treatment depends on benzimidazole-based chemotherapy, targeting the parasite beta-tubulin. Because beta-tubulins are analogous in cestodes and humans, benzimidazoles cannot be used for human treatment even in parasitostatic doses due to their adverse side effects[47]. Direction towards identification of alternative drug targets led to the characterization of nuclear genome sequences of *E. multilocularis* and *E. granulosus* that revealed a large number of targets expressed by the metacestode[48].

Furthermore, recent cell biological investigations demonstrated that *E. multilocularis* employed PSCs, in the germinal cell layer of the cyst wall, which were the only parasite cells capable of proliferation and production of all differentiated cells[49]. It was postulated that because germinative cells are vital for proliferation of *Echinococcus* spp. and for parasite recurrence after failure of chemotherapy, this cellular layer presents a valuable drug target against echinococcosis[47]. Another experimental study on infected rats sought to study the ability of combining BM-MSC transplantation with albendazole therapy on the modulation of immune responses against cyst antigens and the restoration of affected livers. The attempted experiment resulted in the regeneration of injured liver tissue but without complete disappearance of hydatid cyst. Additionally, the host's protective humoral and cell mediated immune responses against hydatid cyst antigens were modulated[50]. The investigators concluded that these results present an opportunity to consider performing clinical trials in humans.

CONCLUDING REMARKS

1. Parasitic diseases have vast impact on patients' lives and on the society. Drugs chemotherapy is fundamentally the choice for the treatment of those parasitic diseases. However, limited drug choices and the continuous emergence of drug resistance makes stem cell therapy a necessity in many parasitic diseases.
2. In experimental schistosomiasis, treatment with BM-MSCs significantly reduced the granuloma size and the expression of alpha-smooth muscle actin (a fibrosis factor) in the hepatic cells.
3. In malaria, experimental treatment with BM-MSCs improved the clearance of parasitized erythrocytes, increased the regeneration of hepatocytes and Kupffer cells and increased the number of astrocytes and oligodendrocytes in the brain.
4. Additionally it has been shown that repeated injections of granulocyte colony-stimulating factor (G-CSF), which mobilizes stem cells from the bone marrow, decreased inflammation and fibrosis in the hearts of chagasic mice.
5. Attempted treatment with MSCs showed a significant regression in the cutaneous lesions produced by *L. major*.
6. In toxoplasmosis, experimental treatment with BM-MSCs combined with SPF showed a significant decrease in the number and size of the mice brain tissue cysts.
7. Recent cell biological investigations demonstrated that *E. multilocularis* employed PSCs, called germinative cells. These germinative cells could be a valuable druggable target against echinococcosis.

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