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Mini Review

Gene-modified mesenchymal stromal cells: A VIP experience

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Administration of *ex vivo* expanded mesenchymal stromal cells (MSCs) represent a promising therapy for degenerative and inflammatory/autoimmune diseases. Indeed, mouse MSCs (mMSCs) and human MSCs (hMSCs) have shown very promising results in animal models for multiple diseases due to their trophic and immunomodulatory activities. However, human clinical trials have not reached the success found in preclinical models. The general consensus is that, for most applications, we should increase the "therapeutic potency" of MSCs before translation into clinic. This goal can be achieved by increasing/improving the migration, engraftment, differentiation and immunomodulatory activities of the MSCs. The present article summarizes some of the approaches that use gene-modified MSCs (GM-MSC) for the treatment of different disorders. We will also discuss our experience using GM-MSCs expressing vasoactive intestinal peptide (VIP) for the treatment of multiple sclerosis.

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Mesenchymal stromal cells for cell therapy

Mesenchymal stromal cells (MSCs) are multipotent cells with self-renewal capacity present in virtually all tissues and that have interesting properties for clinical use: 1- They are ease to isolate and expand, 2- can be differentiated into several tissues, 3- have low immunogenicity, 4- migrate to inflammatory sites, 5- can suppress the immune responses and 6- secrete trophic factors that can help to

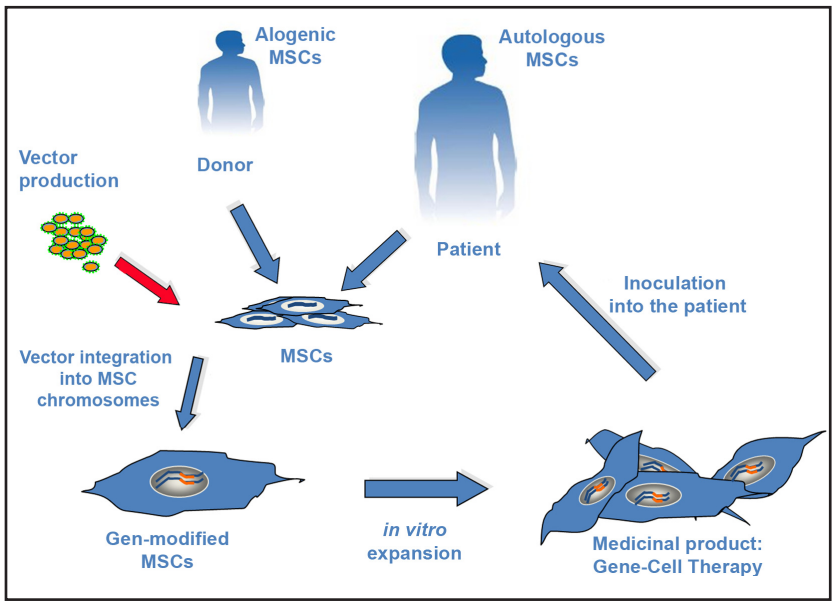

Fig. 1 Cell-gene therapy strategies using gene-modified MSCs

Allogeneic or autologous MSCs can be used for genetic modification using a wide panel of gene therapy vectors (viral and non-viral). However, vector integration is desirable to maintain gene expression during expansion and also for the characterization of the medicinal product. Therefore the use of retroviral (gammaretroviral or lentiviral) vectors is preferred over non-integrative vectors.

the endogenous restoration of different tissues[1]. These properties made the MSCs good candidates for cell therapy of a broad panel of diseases[2-4] most of which have a strong inflammatory/autoimmune component[5].

Bone marrow-derived MSCs (BM-MSCs) were the first MSCs used in preclinical and clinical trials. However, more recently, a broad range of alternative sources such as umbilical cord, endometrial polyps, menses blood, peripheral blood or adipose tissue have been used. Adipose tissue derived MSCs (hASCs) have been widely used during the last years. Their similarity with BM-MSCs, their abundance and their proliferative capacity have made hASCs a promising tool for cell therapy[6-10].

There are at the moment over 370 clinical trials using MSCs of which 27 are in Phase III (data obtained from www.clinicaltrials.gov). However, although several Phase I and II clinical trials rendered very promising results[11-16], a clear demonstration of efficacy in large randomized clinical trials is still lacking[7, 17-21].

Gene transfer into MSCs to improve therapeutic efficacy

Most of the mechanism of action of MSCs are based on secreted factors[22]. In fact for some applications, the use of MSCs-conditioned media has a similar effect compared to the inoculation of MSCs[23, 24]. Therefore, improving/increasing the production of factors involved in its therapeutic activity should be an easy and effective way to increase its potency[25, 26] (Fig. 1). Viral vectors (adenoviral -AdV, adenoassociated-AAV and retroviral-RV) are the most potent system for gene transfer. However, only RVs achieve sustained and stable expression of the transgene on dividing cells and are therefore the vector of choice for MSCs gene manipulation. MSCs modified with RV can be expanded without lost of transgene expression levels and, importantly, they can be characterized before administration into the patient. Non-integrative vectors (AdV or AAV vectors) have also been used to generate GM-MSCs, however, since non-integrative vectors lost the transgene expression upon culture, this strategy will required gene modification of MSCs before infusion into the patient making difficult their characterization for clinical use.

GM-MSCs have been specially studied for the treatment of neuronal diseases[27, 28]. Brain derived neurotrophic factor (BDNF), Glial cell-derived neurotrophic factor (GDNF) and nerve growth factor (NGF) have been expressed in MSCs for the treatment of animal models of Huntington's, Parkinson's and Alzheimer's disease respectively[29-32]. GM-MSCs has also been used for the treatment of spinal cord injury (SCI)[27]. A variety of proteins including neurotrophic factors (NT-3, BDNF, GDNF, HGF, MNTS1), growth factors (HGF, Shh) and kinases (TrKC) have been expressed in MSCs using AdV or RV vectors (reviewed in 27, 33)) with promising results.

Improvement of the therapeutic efficacy of MSCs through genetic manipulation have also been pursued for Diabetes[34], hindlimb ischemia, pulmonary hypertension[35, 36], cardiomyocyte protection[37-39], cerebral ischemia[40], post-infarc-

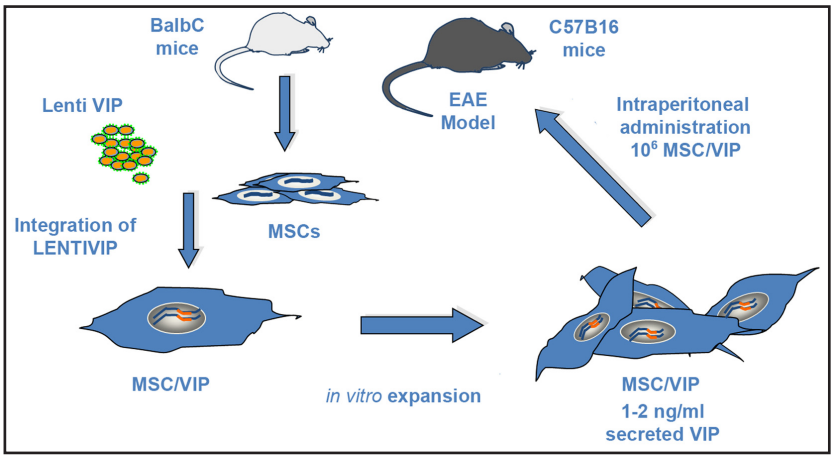

Fig. 2 Cell-gene therapy for the treatment of EAE mice using MSCs/VIP

MSCs were isolated from adipose tissue from BalbC mice. After passage 1, MSCs were transduced with lentiviral vector expressing VIP constitutively (CMV promoter). MSCs/VIP were expanded for 4-6 passages and analyzed for the secretion of bioactive form of the VIP. These allogenic VIP-secreting MSCs were then inoculated into c57b16 mice suffering EAE at the peak of disease mice (MOG model).

tion myocardial repair[41, 42], cartilage repair[43-45] and osteogenesis[46].

MSCs expressing vasoactive intestinal peptide (VIP) for the treatment of multiple sclerosis

Multiple sclerosis (MS) is a severe, demyelinating disorder of the central nervous system (CNS). MS usually begins as a relapsing-remitting (RRMS) disease which in about 65% of the cases, develops into secondary-progressive MS (SPMS). RRMS is characterized by periods of acute disability followed by periods of functional recovery. However, the SPMS phase is characterized by a steady disease progression due to heavy axonal loss and neurodegeneration. In general, currently approved MS therapies (e.g. IFN-beta, glatiramer acetate and mitoxantrone), have shown good therapeutic benefit on RRMS but not on SPMS. In addition they are associated with side effects including depression, multifocal leukoencephalopathy and hypersensitivity reactions.

MSCs were initially thought to be a promising therapy of MS because of their ability to migrate into sites of inflammation, for its immunomodulatory activity and for its ability to enhance neurogenesis. However, although MSCs demonstrated good therapeutic activity of MSCs on the EAE mouse model when treated at early stages[47, 48], human trials were somehow disappointing[49-51]. Therefore, gene modifications of MSCs have been also pursued to increase their therapeutic potential for multiple sclerosis (see Table 1). We hypothesized that increasing the potency of MSCs through forced expression of VIP could be a good strategy. VIP is a neuropeptide of 28 amino acids with strong immunoregulatory (dampening T-cell responses and lowering inflammation)[52, 53] and neuroprotective activities (blocking microglial activation and induction of neuroprotective factors)[54]. Several authors have demonstrated important therapeutic effects of systemic delivery of synthetic VIP in several animal models of autoimmune diseases (Reviewed in 55)) including MS[56-58]. In addition, a phase I/II clinical trial in patients with sarcoidosis demonstrated that inhalation of VIP decreased the levels of inflammatory markers in lung and increased the number of suppressive regulatory T-cells (Tregs)[59]. We therefore generated MSCs/VIP using lentiviral vectors

Table 1 Gene-Cell Therapy approaches for the treatment of experimental autoimmune encephalomyelitis (EAE) using GM-MSCs

Authors	Cell Source	Transplantation	Therapeutic Gene	Animal Model
Ryu CH *et al.*[64]	Bone marrow-derived MSCs (human)	Xenogenic	IFN-β	EAE
Cobo M *et al.*[60]	Adipose-derived MSCs (mice)	Allogenic	VIP	EAE
Payne NL *et al.*[65]	Adipose-derived MSCs (human)	Xenogenic	IL-10	EAE
Payne NL *et al.*[66]	Adipose-derived MSCs (human)	Xenogenic	IL-4	EAE
Mohajeri M *et al.*[67]	Bone marrow-derived MSCs (human)	Xenogenic	FOXP3	EAE
Lu Z *et al.*[68]	Unknown (human)	Xenogenic	CNTF	EAE

that constitutively express the cDNA of VIP gene (Fig. 2). Injection of MSCs/VIP into mice with severe established disease ameliorated disease symptoms while unmodified MSCs had no effect under the same conditions[60]. We will discuss this study in more detail to uncover some of the successes and failures of this approach.

1) Generation of MSCs secreting the bioactive form of VIP: Stability of expression

We routinely generated MSCs that stably secreted of 1-2 ng/ml of the fully processed 3.3kd VIP as detected by ELISA[60]. We could also achieve short term expression of up to 20-30ng/ml of VIP using higher MOIs of LentiVIP. Interestingly, the 19.2-kDa preproVIP form was the main VIP protein secreted and other polypeptides with lower molecular weight were also visible. It is known that both forms (3.3kd and 19.2kd) are bioactive and therefore the secretion of both polypeptides could be an important way to improve bioactivity of the final MSCs/VIP product.

The MSCs/VIP cell lines maintained their main phenotypic characteristics in terms of morphology, differentiation potential and expression of membrane markers. Importantly, the different MSCs/VIP cell lines shared the following properties: 1- The expression of VIP was maintained in MSCs/VIP over time in culture for up to 15 passages. 2- The VIP secreted was more stable than the synthetic VIP peptide. 3- The inmunomodulatory capacity of MSCs/VIP cells was slightly increased compared to MSCs. Therefore by injecting MSCs/VIP we are introducing cells able to migrate to the inflammatory sites, deliver bioactive VIP and, in addition, exert all the therapeutic roles of MSCs but with a better inmunoregulatory activity.

2) MSCs/VIP are more potent than MSCs for the treatment of EAE

Most studies showing therapeutic effect of MSCs on EAE administered the cells at early stages of the disease. However, MSCs do not have significant therapeutic activity when the disease progress toward a more aggressive phenotype[61-63]. We explored the therapeutic potential of MSCs/VIP and MSCs at peak of disease (complete paralysis of back legs and partial paralysis of front legs). Only MSCs/VIP-treated mice recovered from complete hind leg paralysis toward a moderate hind leg paresis whereas the MSCs-treated mice developed a more aggressive disease. None of the MSCs/VIP treated mice required to be sacrificed, while 50%, and 70% of the mice treated with MSCs and PBS respectively were sacrificed due to EAE severity[60].

3) Potential mechanisms behind the therapeutic effects of MSCs/VIP

The aim of using MSCs/VIP for the treatment of severe MS was to deliver VIP and MSCs to the damaged CNS to stop neurodegeneration and inflammation and to favor regeneration. We found that MSCs/VIP treated mice had lower T cell responses, better CNS integrity and reduced astrogiosis compared to MSCs-treated mice. These data indicated that the VIP secreted by MSCs/VIP must be playing a role dampening inflammatory responses and/or protecting damaged neurons. However, the decreased neuronal degeneration not necessarily proves a direct action of VIP in the CNS. Indeed, the improved CNS integrity of MSCs/VIP treated mice could be due to a lower immune attack followed by endogenous repair mechanisms. To demonstrate a direct action of MSCs/VIP in the CNS, over-expression of VIP up-regulated genes (BDNF and ADNP) and decreased expression of VIP down-regulated genes (IL-17, TNF-alpha, IL-6 and iNOS) must be shown. However we found a similar decrease in proinflammatory cytokines in the spinal cords of both MSCs- and MSCs/VIP-treated mice. Similarly IL-10, BDNF and ADNP were also increased in both groups. In addition, only a fraction of the MSCs reached the inflammed CNS while most of the engrafted cells ended in the liver and spleen. Therefore our data pointed to a peripheral effect of MSCs/VIP rather than an effect due to migration of the MSCs/VIP to the damaged CNS. Our theory is that the therapeutic activity of MSCs/VIP relies on their effect in the spleen and lymph nodes. The joint activities of MSCs and VIP will suppress T cell responses, inflammatory macrophages and possibly affect dendritic cells phenotype (interfering with their function). However, a possible effect of MSCs/VIP directly in the damaged CNS cannot be completely eliminated since we found some evidences (although not significant) of VIP activity. For example, we detected a small increase in Foxp3 mRNA in the CNS of mice treated with MSCs/LentiVIP compared with those treated with MSCs controls. It is also possible that the MSCs/VIP reached the CNS but their effect could only be detected during the first days after inoculation. New experiments are on-going in our laboratory to investigate this possibility.

Conclusions Remark

Our experience using GM-MSCs for the treatment of EAE demonstrated that we can improve the therapeutic activity of MSCs without altering their main properties. Indeed, since the main therapeutic effects of MSCs are based on their inmunomodulatory activitity and the secretion of trophic factors, the over-expression of molecules having these effects endorses MSCs with a higher potency. However there still too many unanswered question in terms of mechanism of action, migration potential and durability of the engraftment. In addition, we still must demonstrate that gene manipulation does not have any side effects on MSCs, such as cell transformation. Once we can answer all these questions we will be in a position to translate GM-MSCs into clinic.

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Conflict of interests

None

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