Blackwell Publishing

Endothelium-specific gene and stem cell therapy for ED
DOI: 10.1111/j.1745-7262.2008.00362.x

AJA
www.asiaandro.com

·*Review*·

Endothelium-specific gene and stem cell-based therapy for erectile dysfunction

Travis D. Strong[1], Milena A. Gebska[2], Arthur L. Burnett[1], Hunter C. Champion[2], Trinity J. Bivalacqua[1]

[1]*The Brady Urological Institute, Department of Urology, Johns Hopkins Hospital, Baltimore, MD 21287, USA*
[2]*Division of Cardiology, Department of Medicine, Johns Hopkins Hospital, Baltimore, MD 21287, USA*

Abstract

Erectile dysfunction (ED) commonly results from endothelial dysfunction of the systemic vasculature. Although phosphodiesterase type 5 (PDE-5) inhibitors are effective at treating most cases of ED, they must be taken routinely and are ineffectual for a meaningful number of men. In recent years gene and stem cell-based therapies targeted at the penile endothelium have been gaining momentum in preclinical studies. These early studies reveal that gene and stem cell-based therapies may be both enduring and efficacious, and may eventually lead to a cure for ED. The following review will highlight our current understanding of endothelial-specific gene and stem cell-based therapies performed to date in a number of experimental animal models. *(Asian J Androl 2008 Jan; 10: 14–22)*

Keywords: erectile dysfunction; endothelial-specific gene; endothelial dysfunction; gene therapy

1 Introduction

Erectile dysfunction (ED) is defined as the persistent inability to achieve and maintain an erection of sufficient quality to permit satisfactory sexual intercourse [1]. In recent years, a growing number of studies have elucidated the role of the endothelium in the normal physiology of erections, as well as the pathophysiology of ED [2, 3]. Once believed to serve as only a passive barrier, the endothelium is now considered a primary mediator of vascular blood flow and muscle tone, as directed by neural, humoral, and mechanical stimuli.

As principally a disease of vascular origin, ED correlates highly in men with hypercholesterolemia, cardiovascular disease, hypertension, and diabetes mellitus [4]. Linking these conditions is the presence of endothelial dysfunction, a pathological state of the vasculature involving the loss of the endothelium's responsiveness to vasodilator mediators or an increase in sensitivity to vasoconstrictors. Because ED generally presages or presents concurrently with cardiovascular risk factors, and because cardiovascular disease has been clearly associated with endothelial dysfunction, it is reasonable to infer that ED may result from endothelial dysfunction of the penile vasculature [5, 6]. A number of both clinical and preclinical studies on hypercholesterolemia, hyptertension, diabetes, and aging have demonstrated endothelial dysfunction to be a critical factor in the development of vasculogenic ED [7].

Endothelial dysfunction broadly refers to any pathological condition that inhibits the homeostatic activities of the endothelium, though the term generally connotes an attenuated endothelium-dependent smooth muscle relaxation resulting from diminished nitric oxide (NO) bioavailability within the vasculature. Although ED may be caused by an array of etiologies, endothelial dysfunction plays a preponderant role in a significant number of vasculogenic ED cases. Endothelial dysfunction within the penis is characterized by endothelial NO synthase (eNOS) uncoupling, abnormal eNOS expression and regulation, lack of eNOS substrate or cofactors, and increased oxidative stress with concomitant degradation of NO [8]. Responses to NO donors such as sodium nitroprusside in the presence of endothelial dysfunction demonstrates that the machinery for vasodilation often remains intact and that endothelium-specific aberrations

Correspondence to: Dr Trinity J. Bivalacqua, The Brady Urological Institute, Department of Urology, Johns Hopkins Hospital, 600 N. Wolfe Ave., Marburg 143 Baltimore, MD 21287, USA.
Tel: +1-410-955-0352 Fax: +1-410-614-3695
E-mail: tbivala1@jhmi.edu

are responsible for the diminished bioavailability of NO.

Despite the profound success of current pharmacotherapy for ED, there remains an appreciable contingent of men with intractable ED. The vascular damage accompanying diabetes, for instance, is often too disruptive to endothelial physiology to permit adequate erections, even with pharmacological intervention. It is therefore imperative to find novel ways to combat endothelial-dependent ED. Recently, preclinical studies with gene and stem cell therapies targeting the vascular endothelium in the penis have proven promising (Table 1). It is the intent of this review to survey these early studies and to foster interest into this burgeoning field of research.

2 Gene therapy for ED

The idea of gene therapy arose gradually in the 1960s and 1970s, with the first clinical experiment conducted in 1970 using the Shope papillomavirus to deliver arginase to two girls with arginemia [9]. Like many early attempts at gene therapy, this experiment failed but beckoned in an era of intensive preclinical and clinical evaluations of the concept, leading to the first clinical gene therapy cure in 2000 [10]. Introduction of exogenous genes may be accomplished through a number of vectors ranging from the viral (e.g. retroviruses, adenoviruses [Adv], Sinbisviruses) to the nonviral (e.g. liposomes or naked DNA, gold nanoparticles), though replication-deficient retroviruses, adenoviruses, and adeno-associated viruses (AAV) currently predominate most gene therapy studies in the literature. Primary drawbacks to gene therapy are random transgene expression and insertional mutagenesis.

Owing to its external positioning and limited blood flow that would hinder non-target infection in the systemic circulation, the penis is an amenable structure for gene therapy [11, 12]. In addition, cells within the penis, particularly vascular smooth muscle cells, appear to have a relatively low turnover rate. This may allow long-term expression of introduced genes and thus a more enduring therapeutic option to current pharmacological agents. Another potential benefit of gene therapy for ED is the directed correction of any aberrant biochemical pathway that manifests as penile vascular dysfunction. A number of deviant biochemical pathways are acknowledged to lead to ED, and as long as a therapeutic gene exists that can enhance or replace deficient functions it is theoretically possible to introduce the reparative gene [13]. Currently, gene therapy for ED has emphasized the NO/cGMP/protein kinase G (PKG) pathway, reflecting the principal role of NO bioavailability in the achievement and maintenance of erections [14, 15]. However, a diverse group of introduced genes corresponding to an array of biochemical pathways have shown promising preclinical results as well. In addition, a recent Phase I clinical study has established the safety and apparent efficacy of gene therapy for ED using the human smooth muscle Maxi-K channel [16]. Because the corporal endothelium is a key mediator of penile NO, many gene therapies have focused on repairing deficient endothelial NO production (Figure 1A).

2.1 eNOS gene therapy

Chiefly expressed within the endothelium, eNOS plays a vital role throughout the systemic vasculature in producing NO necessary for smooth muscle relaxation [17]. Several studies have considered eNOS gene therapy for various vascular pathologies with promising results [18–22]. In the penis, eNOS functions to maintain erections by supplying a prolonged release of NO to the overlying smooth muscle cells [2]. Diminished expression or abnormal post-translational modification of eNOS has been documented within the corpora cavernosa in a num-

Table 1. List of endothelium-specific gene and cell-based therapies for erectile dysfunction (ED). AAV, adeno-associated viruses; eNOS, endothelial nitric oxide synthase; EC-SOD, extracellular superoxide dismutase; MSCs, mesenchymal stem cells; VEGF, vascular endothelial growth factor.

Model of ED	Virus or stem cell	Gene	Reference
Aged rat	Adenovirus	*eNOS*	Champion *et al.* [20]
			Bivalacqua *et al.* [23]
Diabetic rat	Adenovirus	*eNOS*	Bivalacqua *et al.* [24]
			Bivalacqua *et al.* [25]
Priapic mice	Adenovirus	*eNOS*	Champion *et al.* [27]
Castrated rats	AAV	*VEGF*	Rogers *et al.* [38]
Hypercholesterolemic rats	Adenovirus	*VEGF*	Ryu *et al.* [39]
Aged rats	Adenovirus	*EC-SOD*	Bivalacqua *et al.* [46]
Diabetic rats	Adenovirus	*EC-SOD*	Bivalacqua *et al.* [51]
Aged rats	MSCs	eNOS-transduced	Bivalacqua *et al.* [77]
Young rats	MSCs	–	Song *et al.* [78]

ber of animal models of aging, hypercholesterolemia, hypertension, and diabetes [8]. Accordingly, attenuated activity of eNOS leads to increased vascular tone and tendencies to developing ED.

The critical role of eNOS in regulating erectile physiology has inspired a number of studies evaluating virally introduced eNOS to the corpora cavernosa. In a rat model of age-related ED, it was found that adenoviral gene transfer of eNOS was able to enhance erectile responses to cavernous nerve stimulation, acetylcholine, and a phosphodiesterase type 5 (PDE-5) inhibitor [20]. From this and other studies, adenoviral overexpression of eNOS increases eNOS mRNA and protein expression, as well as the predominant second messenger cGMP, within the aged penis [23]. Similarly, in a rat model of diabetic-associated ED, intracavernous eNOS transduction resulted in rectified erectile responses to cavernous nerve stimulation and increased corporal NO bioavailability [24]. In an analogous study, Bivalacqua *et al*. [25] demonstrated synergistic effects of intracavernous eNOS transduction and acute systemic administration of the PDE-5 inhibitor sildenafil on increasing erectile function and cGMP levels within diabetic rat penes. Paradoxically, eNOS knockout mice are subject to priapism, a consequence of downregulated PDE-5 expression with the resultant inability to break down even modest levels of cGMP [26, 27]. In an eNOS knockout mouse model of priapism, gene transfer of eNOS resulted in normalized PDE-5 expression and correction of priapic activity, providing an example of the utility of eNOS gene transfer technology to further study endothelial function [27].

2.2 Vascular endothelial growth factor (VEGF) gene therapy

One of the more intriguing determinants of erectile biology is the growth factor, VEGF. VEGF is a critical mediator of endothelial and smooth muscle physiology, and VEGF reduction is associated with a number of pathophysiological changes in the penis. In various models of ED, VEGF, as well as its receptor, flk-1, are down-regulated [28–30]. Given the critical role of VEGF in erectile biology, several studies have considered whether VEGF protein therapy ameliorates vasculogenic ED. In the first study of its kind, Henry *et al*. [31] evaluated the effects of VEGF protein delivery in an atherosclerotic rabbit model of ED and concluded that intracavernous injections of VEGF seemed to protect the penile corporal endothelium against the damaging effects of hypercholesterolemia. Corroborating this result, another study considering hypercholesterolemia-induced ED found evidence of hyperplasia and hypertrophy within the endothelium following intracavernous VEGF protein delivery, suggestive of active angiogenesis [32]. Animals not receiving intracavernous VEGF, however, displayed endothelial denudation and platelet attachment in the sinusoids. Further studies have shown a protective effect of intracavernously delivered VEGF protein in traumatic arteriogenic and diabetic models of ED [33–36].

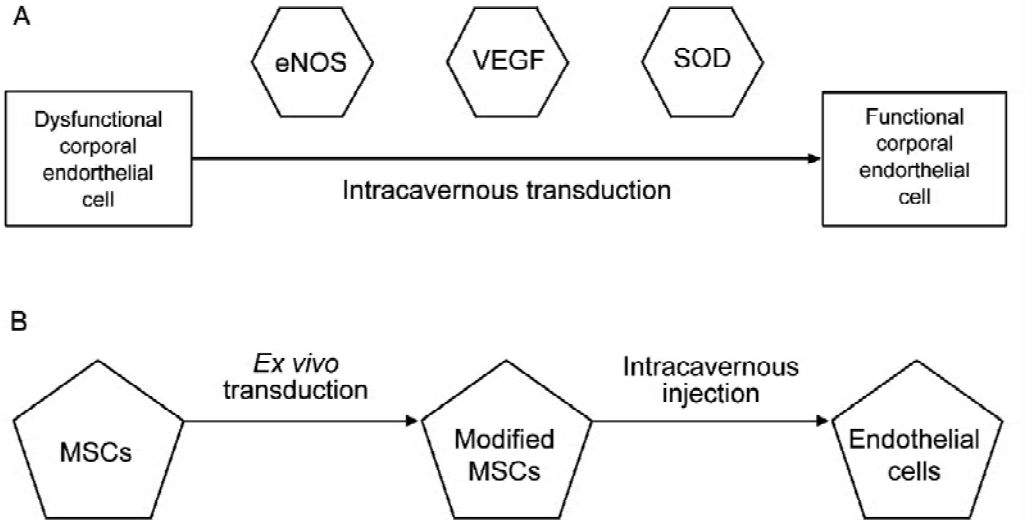

Figure 1. Schematic overview of current preclinical gene- and stem cell-based therapies for treating erectile dysfunction (ED). (A): Intracavernous viral delivery of endothelial nitric oxide synthase (eNOS), vascular endothelial growth factor (VEGF), and extracellular superoxide dismutase (EC-SOD) have delivered critical genes to the corporal endothelium, remedying underlying defects in vasorelaxant pathways and improving erectile function in a number of ED models. (B): Mesenchymal stem cells (MSCs), with or without eNOS transduction, have been isolated from bone marrow of syngeneic rats and injected intracavernously in an aged model of vasculogenic ED, resulting in improved NO bioavailability and erectile function.

These studies and others have largely elucidated the mechanisms by which VEGF exerts its protective effects on the corporal endothelium. Owing to its mitogenic properties, VEGF induces hyperplasia and hypertrophy of endothelial cells, which may counteract the endothelial apoptosis common to some manifestations of ED [34]. VEGF may also directly induce anti-apoptotic pathways within the endothelium [35]. In addition, VEGF protects the endothelial response to acetylcholine, restores levels of sex hormone receptors, increases expression of eNOS, and directs stimulatory eNOS phosphorylation [31, 34, 36, 37].

Built upon the demonstrable evidence that VEGF protein therapy enhances erectile responses in disease models, along with a mechanistic understanding of how VEGF improves endothelial physiology, several recent studies have evaluated VEGF gene therapy for ED. The general intent of these studies was to ascertain whether introduced VEGF DNA would result in the long-term expression of VEGF, perhaps serving as a curative or near curative treatment for vasculogenic ED. In a model of venogenic ED resulting from castration, intracavernous delivery of VEGF mediated by AAV induced endothelial cell hyperplasia and hypertrophy, suggestive of active angiogenesis [38]. Furthermore, intracavernous AAV-VEGF delivery at the time of castration prevented venous leak and ED. Subsequently, it was discovered that intracavernous delivery of VEGF by Adv in a venogenic model of ED increases the phosphorylation of eNOS at Serine 1177, greatly enhancing NO production [37]. Adv-VEGF treated mice also demonstrated a recovery in erectile function. In contrast to wild-type mice, eNOS knockout mice were non-responsive to Adv-VEGF therapy, demonstrating a paramount role of eNOS in mediating the beneficial effects of VEGF. In a recent study of hypercholesterolemia-associated ED, intracavernous Adv-VEGF delivery greatly enhanced endothelial content of the corporal sinusoids. When combined with Adv-angiopoietin-1 (Adv-Ang1), Adv-VEGF markedly increased factor VIII-positive endothelial density within the penes of hypercholesterolemic rats. Additionally, the ratio of phospho-eNOS (Ser1177) to total eNOS was strikingly higher in hypercholesterolemic rats receiving Adv-VEGF or Adv-VEGF + Adv-Ang1 treatment. Underscoring the critical role of the endothelium in erectile physiology, Adv-VEGF alone and combined with Adv-Ang1 significantly increased erectile responses to electrical stimulation [39].

2.3 Extracellular superoxide dismutase (EC-SOD) gene therapy

Oxidative stress has been cogently demonstrated as a mediator of endothelial dysfunction within the systemic vasculature and of erectile dysfunction in the penis [40]. Reactive oxygen species (ROS), most notably superoxide, scavenge NO, forming the highly toxic peroxynitrite. Both superoxide and peroxynitrite serve to uncouple eNOS within the endothelium, which leads to yet further production of ROS. In addition, inducible nitric oxide synthase (iNOS), the Ca-independent NOS isoform, is expressed in endothelial cells under stressed conditions and further contributes to ROS production [41]. Endogenous enzymes, such as NADPH oxidase and xanthine oxidase, expressly produce ROS and are often elevated in diseases associated with endothelial dysfunction. Combating the pernicious effects of ROS are endogenous antioxidants and enzymes such as SOD. SOD catalyzes the dismutation of superoxide into hydrogen peroxide and water and is expressed in a number of cells including the vascular endothelium.

Several studies have considered the potential therapeutic effect of introducing SOD through gene therapy to quench ROS. Virally delivered SOD has yielded therapeutic effects in models of hypertension, hypercholesterolemia, heart failure, and aging [42–45]. Considering that the penile endothelium is a microcosm of the systemic vascular endothelium, a small number of pioneering studies have evaluated the therapeutic potential of intracavernous adenoviral gene transfer of EC-SOD, the predominant SOD isoform found in the vessel wall of the systemic vasculature. In a model of age-related ED in rats, Bivalacqua *et al*. [46] described increased superoxide production, diminished erectile responses to cavernous nerve stimulation and endothelium dependent agonists, elevated nitrotyrosine staining (a measure of oxidative stress), and reduced cGMP production in aged cavernous tissue. However, there was no commensurate increase in EC-SOD to combat the heightened superoxide levels. Upon administration of virally-introduced EC-SOD, EC-SOD mRNA, protein, and activity levels markedly increased. Furthermore, cGMP levels improved while nitrotyrosine staining within the endothelium declined. These molecular data were corroborated by salient improvements in erectile responses to cavernous nerve stimulation, as well as the endothelium-dependent vasodilator acetylcholine, demonstrating that EC-SOD gene therapy may alleviate ROS damage within the penile endothelium and restore erectile function.

Diabetes mellitus is an intricate disease with a constellation of pathophysiological etiologies and sequelae. As with age, strong evidence indicates that enhanced ROS, particularly superoxide, is a critical mediator of diabetes-related vascular dysfunction [47, 48]. Endothelial dysfunction as a result of diabetes in the penile vascular bed may be a result of ROS generation, thus decreasing NO bioavailability [49–51]. Bivalacqua *et al*. [51] attempted to discern whether EC-SOD gene therapy could moderate the levels of superoxide in the diabetic penis, as well as improve physiological measurements of

erectile function. The authors noted that in the diabetic penis, EC-SOD expression was insufficient to reduce superoxide production, and that overexpression of EC-SOD using an adenoviral vector attenuated superoxide levels, increased cGMP production, and significantly improved erectile physiology. These data suggest that reduction of superoxide anion improves erectile physiology through an endothelium-dependent manner secondary to improved corporal cGMP production.

2.4 Molecular targets indirectly affecting penile endothelium

Any agent that acts to increase penile blood flow will indirectly improve corporal endothelial physiology via phosphorylation of the eNOS enzyme and increased production of endothelial-derived NO. A number of gene therapies aimed at correcting neuronal or smooth muscle physiology therefore may improve endothelial function within the penis. Briefly, intracavernous gene delivery of ion channels (maxi-K channel), brain derived neurotrophic factor (BDNF), calcitonin gene-related peptide (CGRP), penile neuronal NOS (PnNOS), vasoactive intestinal peptide (VIP), insulin-like growth factor-1 (IGF-1), and PKG have all demonstrated promising preclinical efficacy at remedying ED and increasing blood flow to the penis [15, 52–57]. Irrespective of the mechanism, increased vascular shear stress resulting from enhanced penile blood flow may lead to phosphorylation of eNOS and improve endothelial derived NO biosynthesis. However, these conclusions are purely speculative at this time and need further investigation. In one of the earliest studies, iNOS was shown to demonstrate improvements to erectile physiology [58]. However, iNOS is not a normal component of penile erection and may lead to increased ROS [59]. Furthermore, inducible NOS (iNOS) has been shown to cause endothelial dysfunction of the penile vascular bed [60].

3 Future of gene therapy for ED

Although the present preclinical gene therapy studies have demonstrated efficacy, there are a number of drawbacks that may limit their clinical implementation. First, the viral vectors evaluated so far run the risk of random transgene expression throughout the systemic vasculature. Because the vectors have demonstrated the capacity for transcytosis, virtually every cell within the body may be susceptible to transduction. Even within the penis, it would be beneficial to specify the transduction of a particular cell phenotype, such as endothelial cells or neurons—the vectors evaluated so far have demonstrated ecumenical transduction of diverse penile cells. Second, owing to the costs involved in producing viral vectors, it would be beneficial to find a way to limit the amount of virus required to efficaciously transduce penile cells. Limiting viral load requirements may also diminish the risks of inflammation and resultant fibrosis.

Recent advancements in targeting viral vectors to specific cell phenotypes may provide a solution to the above concerns. In contrast to a virus that can transduce an array of cell types, the specificity of these vectors can be finely adjusted so that expression occurs within a much more circumscribed set of cell types. If specificity can be fine-tuned, it may become possible to administer vectors systemically through intravenous injection. The viral vectors could then target appropriate cells without damaging non-targeted cells. Transcriptional targeting has been advocated as one such method of effecting specificity [61]. With transcriptional targeting, promoters are selected that are uniquely expressed within a particular cell type. That is, if smooth muscle cells are targeted, then viral genes could be linked to the promoter of desmin or some similar muscle-specific promoter. The more restrictive the promoter, the more specific the expression will be.

Alternatively, specificity could be realized through transductional targeting [62]. With transductional targeting, viral vectors are directed to transduce only those cell types that express certain membrane markers. Using this technique, it may be possible to preclude the undesired transduction of non-targeted cells. Several strategies have been suggested for achieving transductional targeting *in vivo*, including pseudotyping, adaptor systems, and genetic systems [63–65]. Concerning potential therapies for ED, it may be possible to devise a virus that specifically transduces target cells, thus lowering required viral load and the risk of random transgene expression. Such a highly specified vector would be desirable for systemic delivery. Even if delivery continued to be through intracavernous injection, a virus that was either transcriptionally or transductionally targeted may permit a more physiologically normal distribution of gene expression. Increased specificity of cell type expression may yield more natural responses to physiological stimuli, thus enabling more normal erection physiology.

The true potential of gene therapy to mitigate or even cure ED remains to be rigorously tested in a clinical setting. Nevertheless, a very promising Phase I clinical trial using plasmid-based gene delivery has shown that gene therapy to the penis may be both safe and effective in humans [16]. Combined with the flurry of preclinical studies with viral-based gene therapy for ED, pioneering studies such as this may lead to yet more efficacious treatments for ED in the future.

4 Stem cell primer

Stem cells have garnered significant attention recently

for their suspected potential to treat currently intractable diseases. While some of the more extravagant claims are hyperbolic and excessively optimistic, there is clear validity to the notion that stem cells may lead to novel and potentially curative therapies. Resulting from age and degenerative diseases, cells may wear out, becoming dysfunctional or succumbing to apoptosis or necrosis. Indeed, the etiological basis for several prominent diseases, such as diabetes mellitus, sarcopenia of aging, heart failure, and Parkinson's disease, is cell dysfunction or loss [66–69]. For these and numerous other maladies, pharmacotherapy may not be able to induce the regenerative capacity of the remaining tissue. Ostensibly, many conditions will benefit from a treatment strategy that either transplants donor cells or enhances the proliferative potential of tissue-resident stem cells, thus functionally restoring the damaged tissues.

Although variably defined, stem cells are generally attributed the capacity for 1) self-renewal, 2) differentiation into one or more distinct phenotypes, and 3) functional reproduction of damaged tissues [70]. Varying stem cell populations fall along a gradient of differentiation potential from those cells capable of becoming any cell within the organism (pluripotent), to those with more limited differentiation potential yet able of becoming several distinct cell types (multipotent), to those with the potential of becoming only one or a small number of cell types (progenitor) [71–73]. While embryonic stem cells within the inner cell mass are currently the only well-acknowledged pluripotent stem cells, the adult human has a number of multipotent and progenitor cell populations residing within various tissues and organs for the regular replacement of lost or failing cells [74].

5 Stem cell therapy for ED

Currently, only a very small number of studies have evaluated the prospects of using stem cells to treat ED [75–78], and cell-based therapy in general for treating ED is quite new [79]. However, the potentially curative nature of such treatments will undoubtedly encourage a spate of forthcoming studies with varying stem cell populations and strategies. Conceivably, stem cell therapies may reasonably obviate the need to understand the intricate molecular bases underlying ED. Current pharmacological agents target precise biochemical pathways within the penis that are aberrantly regulated under certain disease states. Because there are a myriad of potential mechanistic causes of ED, pharmacotherapy must be able to treat each etiology uniquely by correcting specific deviant pathways. By wholly replacing the operative responsibilities of dysfunctional cells, repairing specific molecular pathologies may not be critical. It is certainly an oversimplification to discount the benefits of elucidating the detailed molecular bases of ED, but much of the exciting potential of stem cells rests in their demonstrable capacity to either take over the function of lost or damaged cells or secrete compounds in situ that in some way normalize dysfunctional cells. Because endothelial dysfunction of the penile vasculature is a common cause of vasculogenic ED, rescuing the penile endothelium with transplanted stem or progenitor cells may functionally restore normal erectile responses in a large number of ED cases. Presently, only mesenchymal stem cells have been evaluated for their potential to treat vasculogenic ED (Figure 1B) [77].

5.1 MSCs

Residing within the bone marrow and a number of other tissue and organ reservoirs is a rare population of multipotent stem cells called MSCs, which are uniquely able to differentiate into a diverse array of cellular phenotypes [80–84]. Because of their combined virtues of robust proliferation, multipotency, and susceptibility to genetic manipulation, MSCs have been studied vigorously for their therapeutic potential. In preclinical studies, MSCs have shown the ability to home to and repair damaged tissues [85, 86], and clinical studies are currently monitoring their capacity to remedy a number of pathological conditions [87].

Given the right environment, MSCs have the established capacity to differentiate into diverse cell types and thus conceivably may be able to replace damaged or dysfunctional tissues of the penis. MSCs can express endothelial and smooth muscle phenotypes *in vitro* and have been used successfully to repair vascular diseases and insults *in vivo*, suggesting the possibility that this stem cell population may be effective in replacing or rejuvenating the dysfunctional analogous tissues within the penis [88–90].

At the present time, only two published studies are known to have evaluated the potential for using MSCs to treat vasculogenic ED [77, 78]. In the first of these studies, Bivalacqua *et al*. [77] investigated the potential of MSCs to differentiate into new endothelial cells and improve endothelial-derived NO bioavailability and erectile physiology. This and previous studies have documented that rat MSCs can be readily transduced with an Adv expressing eNOS and consequently express long-term high levels of eNOS protein, eNOS activity, and cGMP concentrations [91, 92]. Aged rats with demonstrable vasculogenic ED and reduced NO bioavailability were given intracavernous injections of MSCs with or without transduction with eNOS. Seven days after injection the MSCs were adhered to the endothelium, and after twenty-one days many of the cells were expressing endothelial antigens. eNOS expression/activity, cGMP, and erectile responses were significantly increased within the penis after twenty-one days in both the normal and

transduced MSCs, suggesting the long-term capacity of these cells to replace dysfunctional endothelial cells. In addition, no signs of inflammation were noted at 7 and 21 days following injection of MSCs.

Subsequently, Song *et al*. [78] evaluated the potential of immortalized human fetus-derived MSCs to differentiate into endothelial and smooth muscle cells within rat corpora cavernosa. The immortalized MSCs were injected into the cavernosa of young, healthy rats, and two weeks later the penes were collected and studied for endothelial and smooth muscle antigens. Histological assessment of the penes revealed the expression of endothelial-specific antigens within the corpora, suggesting that many of the MSCs had differentiated into endothelial-like cells. Considered together, these two early studies appear to provide evidence for the potential of MSCs from various sources to differentiate into fully functional endothelial cells and palliate both endothelial-dependent NO bioavailability and erectile responses.

6 Conclusion

As the primary mediator of NO-derived smooth muscle relaxation, the penile endothelium is critical for normal erectile physiology. Although PDE-5 inhibitors are highly efficacious for a majority of men with ED, a large contingent are unresponsive or have strict contraindications to the drug and require alternative treatment options. In addition, a drawback to oral pharmacotherapy is the requirement for routine administration. Preclinical gene and stem cell-based therapies demonstrate apparent long-term efficacy in animal models. Already, gene therapy for ED is being evaluated in clinical trials, and stem cell-based approaches will likely follow. Combined with the remarkable success of PDE-5 inhibitors, endothelium-specific gene and stem cell-based therapies may someday add to our growing armamentarium against ED.

References

1 NIH Consensus Conference. Impotence. NIH Consensus Development Panel on Impotence. JAMA 1993; 270: 83–90.
2 Hurt KJ, Musicki B, Palese MA, Crone JK, Becker RE, Moriarity JL, *et al*. Akt-dependent phosphorylation of endothelial nitric-oxide synthase mediates penile erection. Proc Natl Acad Sci USA 2002; 99: 4061–6.
3 Bivalacqua TJ, Usta MF, Champion HC, Kadowitz PJ, Hellstrom WJ. Endothelial dysfunction in erectile dysfunction: role of the endothelium in erectile physiology and disease. J Androl 2003; 24: S17–37.
4 Burnett AL. Erectile dysfunction. J Urol 2006; 175: S25–31.
5 Maas R, Schwedhelm E, Albsmeier J, Boger RH. The pathophysiology of erectile dysfunction related to endothelial dysfunction and mediators of vascular function. Vasc Med 2002; 7: 213–25.
6 Solomon H, Man JW, Jackson G. Erectile dysfunction and the cardiovascular patient: endothelial dysfunction is the common denominator. Heart 2003; 89: 251–3.
7 Watts GF, Chew KK, Stuckey BG. The erectile-endothelial dysfunction nexus: new opportunities for cardiovascular risk prevention. Nat Clin Pract Cardiovasc Med 2007; 4: 263–73.
8 Musicki B, Burnett AL. eNOS function and dysfunction within the penis. Exp Biol Med 2006; 231: 154–65.
9 Rogers S. Reflections on issues posed by recombinant DNA molecule technology. II. In: Lappe M, Morison RS, editors. Ethical and Scientific Issues Posed by Human Uses of Molecular Genetics. New York: New York Academy of Sciences; 1976. p66–70.
10 Cavazzana-Calvo M, Hacein-Bey S, de Saint Basile G, Gross F, Yvon E, Nusbaum P, *et al*. Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. Science 2000; 288: 669–72.
11 Christ GJ, Melman A. The application of gene therapy to the treatment of erectile dysfunction. Int J Impot Res 1998; 10: 111–2.
12 Bivalacqua TJ, Hellstrom WJ. Potential application of gene therapy for the treatment of erectile dysfunction. J Androl 2001; 22: 183–90.
13 Kendirci M, Teloken PE, Champion HC, Hellstrom WJ, Bivalacqua TJ. Gene therapy for erectile dysfunction: fact or fiction? Eur Urol 2006; 50: 1208–22.
14 Bivalacqua TJ, Musicki B, Usta MF, Champion HC, Kadowitz PJ, Burnett AL, *et al*. Endothelial nitric oxide synthase gene therapy for erectile dysfunction. Curr Pharm Des 2005; 11: 4059–67.
15 Bivalacqua TJ, Kendirci M, Champion HC, Hellstrom WJ, Andersson KE, Hedlund P. Dysregulation of cGMP-dependent protein kinase 1 (PKG-1) impairs erectile function in diabetic rats: influence of in vivo gene therapy of PKG1alpha. BJU Int 2007; 99: 1488–94.
16 Melman A, Bar-Chama N, McCullough A, Davies K, Christ G. hMaxi-K gene transfer in males with erectile dysfunction: results of the first human trial. Hum Gene Ther 2006; 17: 1165–76.
17 Shaul PW. Regulation of endothelial nitric oxide synthase: location, location, location. Annu Rev Physiol 2002; 64: 749–74.
18 Lin CJ, Chao L, Chao J. Prolonged reduction of high blood pressure with human nitric oxide synthase gene delivery. Hypertension 1997; 30: 307–13.
19 Varenne O, Pislaru S, Gillijns H, Van Pelt N, Gerard RD, Zoldhelyi, P, *et al*. Local adenovirus-mediated transfer of human endothelial nitric oxide synthase reduces luminal narrowing after coronary angioplasty in pigs. Circulation 1998; 98: 919–26.
20 Champion HC, Bivalacqua TJ, D'Souza FM, Ortiz LA, Jeter JR, Toyoda K, *et al*. Gene transfer of endothelial nitric oxide synthase to the lung of the mouse *in vivo*. Effect on agonist-induced and flow-mediated vascular responses. Circ Res 1999; 84: 1422–32.
21 Champion HC, Bivalacqua TJ, Greenberg SS, Giles TD, Hyman AL, Kadowitz PJ. Adenoviral gene transfer of endothelial nitric-oxide synthase (eNOS) partially restores normal pulmonary arterial pressure in eNOS-deficient mice. Proc Natl Acad Sci USA 2002; 99: 13248–53.
22 Cooney R, Hynes SO, Sharif F, Howard L, O'Brien T. Effect of gene delivery of NOS isoforms on intimal hyperplasia and endothelial regeneration after balloon injury. Gene Ther 2007; 14: 396–404.
23 Bivalacqua TJ, Champion HC, Mehta YS, Abdel-Mageed AB, Sikka SC, Ignarro LJ, *et al*. Adenoviral gene transfer of endothelial nitric oxide synthase (eNOS) to the penis improves age-related erectile function in the rat. Int J Impot Res 2000; 12: S8–17.
24 Bivalacqua TJ, Usta MF, Champion HC, Adams D, Namara DB, Abdel-Mageed AB, *et al*. Gene transfer of endothelial nitric oxide synthase partially restores nitric oxide synthesis and erectile function in streptozotocin diabetic rats. J Urol 2003; 169: 1911–7.
25 Bivalacqua TJ, Usta MF, Champion HC, Leungwattanakij S, Dabisch PA, McNamara DB, *et al*. Effect of combination endothelial nitric oxide synthase gene therapy and sildenafil on erectile function in diabetic rats. Int J Impot Res 2004; 16: 21–9.
26 Burnett AL, Chang AG, Crone JK, Huang PL, Sezen SE. Noncholinergic penile erection in mice lacking the gene for endothelial nitric oxide synthase. J Androl 2002; 23: 92–7.

27 Champion HC, Bivalacqua TJ, Takimoto E, Kass DA, Burnett AL. Phosphodiesterase-5A dysregulation in penile erectile tissue is a mechanism of priapism. Proc Natl Acad Sci USA 2005; 102: 1661–6.
28 Young LD, Bella A, Howard J, Brock G. Arteriogenic erectile dysfunction alters protein expression within the cavernosal tissue in an animal model. J Sex Med 2005; 2: 199–206.
29 Xie D, Kontos CD, Donatucci CF, Annex BH. Cholesterol feeding reduces vascular endothelial growth factor signaling in rabbit corporal tissues. J Sex Med 2005; 2: 634–40.
30 Ryu JK, Shin HY, Song SU, OH SM, Piao S, Han JY, *et al*. Downregulation of angiogenic factors and their downstream target molecules affects the deterioration of erectile function in a rat model of hypercholesterolemia. Urology 2006; 67: 1329–34.
31 Henry GD, Byrne R, Hunyh TT, Abraham V, Annex BH, Hagen PO, *et al*. Intracavernosal injections of vascular endothelial growth factor protects endothelial dependent corpora cavernosal smooth muscle relaxation in the hypercholesterolemic rabbit: a preliminary study. Int J Impot Res 2000; 12: 334–9.
32 Gholami SS, Rogers R, Chang J, HO HC, Grazziottin T, Lin CS, *et al*. The effect of vascular endothelial growth factor and adeno-associated virus mediated brain derived neurotrophic factor on neurogenic and vasculogenic erectile dysfunction induced by hyperlipidemia. J Urol 2003; 169: 1577–81.
33 Lee MC, El-Sakka AI, Graziottin TM, Ho HC, Lin CS, Lue TF. The effect of vascular endothelial growth factor on a rat model of traumatic arteriogenic erectile dysfunction. J Urol 2002; 167: 761–7.
34 Lin CS, Ho HC, Chen KC, Lin G, Nunes L, Lue TF. Intracavernosal injection of vascular endothelial growth factor induces nitric oxide synthase isoforms. BJU Int 2002; 89: 955–60.
35 Yamanaka M, Shirai M, Shiina H, Tanaka Y, Enokida H, Tsujimura A, *et al*. Vascular endothelial growth factor restores erectile function through inhibition of apoptosis in diabetic rat penile crura. J Urol 2005; 173: 318–23.
36 Shirai M, Yamanaka M, Shiina H, Igawa M, Kawakami T, Ishii N, *et al*. Vascular endothelial growth factor restores erectile function through modulation of the insulin-like growth factor system and sex hormone receptors in diabetic rat. Biochem Biophys Res Commun 2006; 341: 755–62.
37 Musicki B, Palese MA, Crone JK, Burnett AL. Phosphorylated endothelial nitric oxide synthase mediates vascular endothelial growth factor-induced penile erection. Biol Reprod 2004; 70: 282–9.
38 Rogers RS, Graziottin TM, Lin CS, Kan YW, Lue TF. Intracavernosal vascular endothelial growth factor (VEGF) injection and adeno-associated virus-mediated VEGF gene therapy prevent and reverse venogenic erectile dysfunction in rats. Int J Impot Res 2003; 15: 26–37.
39 Ryu JK, Cho CH, Shin HY, Song SU, Oh SM, Lee M, *et al*. Combined angiopoietin-1 and vascular endothelial growth factor gene transfer restores cavernous angiogenesis and erectile function in a rat model of hypercholesterolemia. Mol Ther 2006; 13: 705–15.
40 Agarwal A, Nandipati KC, Sharma RK, Zippe CD, Raina R. Role of oxidative stress in the pathophysiological mechanism of erectile dysfunction. J Androl 2006; 27: 335–47.
41 Xu J, He L, Ahmed SH, Chen SW, Goldberg MP, Beckman JS, *et al*. Oxygen-glucose deprivation induces inducible nitric oxide synthase and nitrotyrosine expression in cerebral endothelial cells. Stroke 2000; 31: 1744–51.
42 Fennell JP, Brosnan MJ, Frater AJ, Hamilton CA, Alexander MY, Nicklin SA, *et al*. Adenovirus-mediated overexpression of extracellular superoxide dismutase improves endothelial dysfunction in a rat model of hypertension. Gene Ther 2002; 9: 110–7.
43 Zanetti M, Sato J, Jost CJ, Gloviczki P, Katusic ZS, O'Brien T. Gene transfer of manganese superoxide dismutase reverses vascular dysfunction in the absence but not in the presence of atherosclerotic plaque. Hum Gene Ther 2001; 12: 1407–16.
44 Iida S, Chu Y, Francis J, Weiss RM, Gunnett CA, Faraci FM, *et al*. Gene transfer of extracellular superoxide dismutase improves endothelial function in rats with heart failure. Am J Physiol Heart Circ Physiol 2005; 289: H525–32.
45 Brown KA, Chu Y, Lund DD, Heistad DD, Faraci FM. Gene transfer of extracellular superoxide dismutase protects against vascular dysfunction with aging. Am J Physiol Heart Circ Physiol 2006; 290: H2600–5.
46 Bivalacqua TJ, Armstrong JS, Biggerstaff J, Abdel-Mageed AB, Kadowitz PJ, Hellstrom WJ. Gene transfer of extracellular SOD to the penis reduces superoxide and improves erectile function in aged rats. Am J Physiol Heart Circ Physiol 2003; 284: H1408–21.
47 De Vriese AS, Verbeuren TJ, Van de Voorde J, Lameire NH, Vanhoutte PM. Endothelial dysfunction in diabetes. Br J Pharmacol 2000; 130: 963–74.
48 Channon KM, Guzik TJ. Mechanisms of superoxide productin in human blood vessels: Relationship to endothelial dysfunction, clinical and genetic risk factors. J Physiol Pharmacol 2002; 53: 515–24.
49 Akingba AG, Burnett AL. Endothelial nitric oxide synthase protein expression, localization, and activity in the penis of the alloxan-induced diabetic rat. Mol Urol 2001; 5: 189–97.
50 Podlasek CA, Zelner DJ, Bervig TR, Gonzalez CM, McKenna KE, McVary KT. Characterization and localization of nitric oxide synthase isoforms in the BB/WOR diabetic rat. J Urol 2001; 166: 746–55.
51 Bivalacqua TJ, Usta MF, Kendirci M, Pradhan L, Alvarez X, Champion HC, *et al*. Superoxide anion production in the rat penis impairs erectile function in diabetes: influence of in vivo extracellular superoxide dismutase gene therapy. J Sex Med 2005; 2: 187–97.
52 Christ GJ, Rehman J, Day N, Salkoff L, Valcic M, Melman A, *et al*. Intracorporal injection of hSlo cDNA in rats produces physiologically relevant alterations in penile function. Am J Physiol 1998; 275: H600–8.
53 Bakircioglu ME, Lin CS, Fan P, Sievert KD, Kan YW, Lue TF. The effect of adeno-associated virus mediated brain derived neurotrophic factor in an animal model of neurogenic impotence. J Urol 2001; 165: 2103–9.
54 Bivalacqua TJ, Champion HC, Abdel-Mageed AB, Kadowitz PJ, Hellstrom WJ. Gene transfer of prepro-calcitonin gene-related peptide restores erectile function in the aged rat. Biol Reprod 2001; 65: 1371–7.
55 Magee TR, Ferrini M, Garban HJ, Vernet D, Mitani K, Rajfer J, *et al*. Gene therapy of erectile dysfunction in the rat with penile neuronal nitric oxide synthase. Biol Reprod 2002; 67: 20–8.
56 Shen ZJ, Wang H, Lu YL, Zhou XL, Chen SW, Chen ZD. Gene transfer of vasoactive intestinal polypeptide into the penis improves erectile response in the diabetic rat. BJU Int 2005; 95: 890–4.
57 Pu XY, Hu LQ, Wang HP, Luo YX, Wang XH. Improvement in erectile dysfunction after insulin-like growth factor-1 gene therapy in diabetic rats. Asian J Androl 2007; 9: 83–91.
58 Garban H, Marquez D, Magee T, Moody J, Rajavashisth T, Rodriguez JA, *et al*. Cloning of rat and human inducible penile nitric oxide synthase. Application for gene therapy of erectile dysfunction. Biol Reprod 1997; 56: 954–63.
59 Gonzalez-Cadavid NF, Rajfer J. The pleiotropic effects of inducible nitric oxide synthase (iNOS) on the physiology and pathology of penile erection. Curr Pharm Des 2005; 11: 4041–6.
60 Wessels H, Teal TH, Luttrell IP, Sullivan CJ. Effect of endothelial cell-based iNOS gene transfer on cavernosal eNOS expression and mouse erectile responses. Int J Impot Res 2006; 18: 438–45.
61 Sadeghi H, Hitt MM. Transcriptionally targeted adenovirus vectors. Curr Gene Ther 2005; 5: 411–27.
62 Waehler R, Russell SJ, Curiel DT. Engineering targeted viral vectors for gene therapy. Nat Rev Genet 2007; 8: 573–87.

63 Cronin J, Zhang XY, Reiser J. Altering the tropism of lentiviral vectors through pseudotyping. Curr Gene Ther 2005; 5: 387–98.
64 Pereboev AV, Nagle JM, Shakhmatov MA, Triozzi PL, Matthews QL, Kawakami Y, *et al*. Enhanced gene transfer to mouse dendritic cells using adenoviral vectors coated with a novel adapter molecule. Mol Ther 2004; 9: 712–20.
65 Mahasreshti PJ, Kataram M, Wu H, Yalavarthy LP, Carey D, Fisher PB, *et al*. Ovarian cancer targeted adenoviral-mediated mda-7/IL-24 gene therapy. Gynecol Oncol 2006; 100: 521–32.
66 Lee SC, Pervaiz S. Apoptosis in the pathophysiology of diabetes mellitus. Int J Biochem Cell Biol 2007; 39: 497–504.
67 Marzetti E, Leeuwenburgh C. Skeletal muscle apoptosis, sarcopenia and frailty at old age. Exp Gerontol 2006; 41: 1234–8.
68 Grazzette LP, Rosenzweig A. Role of apoptosis in heart failure. Heart Fail Clin 2005; 1: 251–61.
69 Bredesen DE, Rao RV, Mehlen P. Cell death in the nervous system. Nature 2006; 443: 796–802.
70 Morrison SJ, Shah NM, Anderson DJ. Regulatory mechanisms in stem cell biology. Cell 1997; 88: 287–98.
71 Hoffman JA, Merrill BJ. New and renewed perspectives on embryonic stem cell pluripotency. Front Biosci 2007; 12: 3321–32.
72 Spangrude GJ, Heimfeld S, Weissman IL. Purification and characterization of mouse hematopoietic stem cells. Science 1988; 241: 58–62.
73 Weissman IL, Anderson DJ, Gage F. Stem and progenitor cells: origins, phenotypes, lineage commitments, and transdifferentiations. Annu Rev Cell Dev Biol 2001; 387–403.
74 Harley CB, Rao MS. Human embryonic vs adult stem cells for transplantation therapies. In: Chiu AY, Rao MS, editors. Human Embryonic Stem Cells. Totowa: Humana Press; 2003. p239–64.
75 Bochinski D, Lin GT, Nunes L, Carrion R, Rahman N, Lin CS, *et al*. The effect of neural embryonic stem cell therapy in a rat model of cavernosal nerve injury. BJU Int 2004; 94: 904–9.
76 Kim Y, de Miguel F, Usiene I, Kwon D, Yoshimura N, Huard J, *et al*. Injection of skeletal muscle-derived cells into the penis improves erectile function. Int J Impot Res 2006; 18: 329–34.
77 Bivalacqua TJ, Deng W, Kendirci M, Usta MF, Robinson C, Taylor BK, *et al*. Mesenchymal stem cells alone or *ex vivo* gene modified with endothelial nitric oxide synthase reverse age-associated erectile dysfunction. Am J Physiol Heart Circ Physiol 2007; 292: H1278–H90.
78 Song YS, Lee HJ, Park IH, Kim WK, Ku JH, Kim SU. Potential differentiation of human mesenchymal stem cell transplanted in rat corpus cavernosum toward endothelial or smooth muscle cells. Int J Impot Res 2007; 19: 378–85.
79 Wessels H, Williams SK. Endothelial cell transplantation into the corpus cavernosum: moving towards cell-based gene therapy. J Urol 1999; 162: 2162–4.
80 Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, *et al*. Multilineage potential of adult human mesenchymal stem cells. Science 1999; 284: 143–7.
81 Kopen G, Prockop D, Phinney D. Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. Proc Natl Acad Sci U S A 1999; 96: 10711–6.
82 Ortiz LA, Gambelli F, McBride C, Gaupp D, Baddoo M, Kaminski N, *et al*. Mesenchymal stem cell engraftment in lung is enhanced in response to bleomycin exposure and ameliorates its fibrotic effects. Proc Natl Acad Sci U S A 2003; 100: 8407–11.
83 Aurich I, Mueller LP, Aurich H, Leutzkendorf J, Tisljar K, Dollinger MM, *et al*. Functional integration of hepatocytes derived from human mesenchymal stem cells into mouse livers. Gut 2007; 56: 405–15.
84 da Silva Mereilles L, Chagastelles PC, Nardi NB. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. J Cell Sci 2006; 119: 2204–13.
85 Jiang W, Ma A, Wang T, Han K, Liu Y, Zhang Y, *et al*. Homing and differentiation of mesenchymal stem cells delivered intravenously to ischemic myocardium *in vivo*: a time-series study. Pflugers Arch 2006; 453: 43–52.
86 Nagaya N, Fujii T, Iwase T, Ohgushi H, Itoh T, Uematsu M, *et al*. Intravenous administration of mesenchymal stem cells improves cardiac function in rats with acute myocardial infarction through angiogenesis and myogenesis. Am J Physiol Heart Circ Physiol 2004; 287: H2670–6.
87 Giordano A, Galderisi U, Marino IR. From the laboratory bench to the patient's bedside: an update on clinical trials with mesenchymal stem cells. J Cell Physiol 2007; 211: 27–35.
88 Wu X, Huang L, Zhou Q, Song Y, Li A, Jin J, *et al*. Mesenchymal stem cells participating in ex vivo endothelium repair and its effect on vascular smooth muscle cells growth. Int J Cardiol 2005; 105: 274–82.
89 Wang T, Xu Z, Jiang W, Ma A. Cell-to-cell contact induces mesenchymal stem cell to differentiate into cardiomyocyte and smooth muscle cell. Int J Cardiol 2006; 109: 74–81.
90 Kim SW, Han H, Chae GT, Lee SH, Bo S, Yoon JH, *et al*. Successful stem cell therapy using umbilical cord blood-derived multipotent stem cells for Buerger's disease and ischemic limb disease animal model. Stem Cells 2006; 24: 1620–6.
91 Deng W, Bivalacqua TJ, Chattergoon NN, Hyman AL, Jeter JR Jr., Kadowitz PJ. Adenoviral gene transfer of eNOS: high-level expression in ex vivo expanded marrow stromal cells. Am J Physiol Cell Physiol 2003; 285: C1322–9.
92 Bivalacqua TJ, Deng W, Champion HC, Hellstrom WJ, Kadowitz PJ. Gene therapy techniques for the delivery of endothelial nitric oxide synthase to the corpora cavernosa for erectile dysfunction. Methods Mol Biol 2004; 279: 173–85.