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Review Article

Regenerative medicine for male infertility: A focus on stem cell niche injury models

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ABSTRACT

Stem and progenitor cells located within stem cell niches maintain the renewal and regeneration of tissues and organs throughout the life of an adult organism. Stem cell niche component dysfunction might alter the activity of stem cells and ultimately lead to the development of difficult-to-treat chronic or acute disorders. Of note, some cases of idiopathic male infertility, a highly prevalent diagnosis with no specific treatment options, might be associated with a spermatogonial stem cell(SSC) niche disturbance. To overcome this disease entity, approaches aiming at launching the regeneration of an altered stem cell niche are worth considering. Particularly, mesenchymal stromal cells (MSCs) or their secretome might fulfill this task due to their promising contribution in recovering injured stem cell niches. However, the successful application of MSC-based treatment is limited by the uncovered mechanisms of action of MSCs and their secretome. Specific animal models should be developed or adapted to reveal the role of MSCs and their secretome in a stem cell niche recovery. In this review, in a bid to consider MSCs and their secretome as a therapeutic regenerative approach for idiopathic male infertility we focus on the rationale of SSC niche injury modeling.

Throughout life, the cellular components of tissues and organs need timely renewal. To maintain this renewal, a pool of stem and progenitor cells is present in adult organisms [1]. Their activity of stem and progenitor cells is fine-tuned by a stem cell niche [2], a complex microenvironment of cells that interact via paracrine communication, metabolic, physical, and chemical cues. A dysregulation of stem cell niche components might alter the activity of stem cells and ultimately manifest as severe chronic or acute diseases [3,4]. Usually, given that the choice of therapeutic options is complicated by multiple mechanisms involved in niche dysfunction, these conditions are difficult to treat [5]. An example of such are some cases of idiopathic male infertility, which still lack effective therapeutic approaches despite its high worldwide prevalence [6–8].

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Unlike some other niches, spermatogonial stem cell(SSC) niche cells are less capable of adopting new fates [9,10] that might provoke disease if any highly specialized component is altered. Therefore, to restore spermatogenesis, a complete restoration of the stem cell niche is required. Among regenerative medicine approaches, using of multipotent mesenchymal stromal cells (MSCs) might be an option. MSCs can replenish altered niche components through various mechanisms as well as mimic paracrine signals from adjacent niche cells to guarantee functional niche integrity during regeneration [11]. The effects of MSCs and their secretome have been investigated for the treatment of various disorders of tissue repair and regeneration, and several clinical trials have proven their efficacy [12–14]. However, their application to the restoration of stem cell niche disorders is yet far from being successful. One possible explanation could be the high variability of MSC therapeutic effects determined by the tissue source, donor parameters, approaches to manufacturing, route of delivery as well as the lack of clear characteristic criteria of MSC-based cell products [15–17]. The need to control the proportion of senescent/dysfunctional MSCs before injection [18] and maintain their viability after injection also complicates their effective use [19]. The risks of unwanted differentiation of injected cells, the formation of tumors and ischemic disorders due to the microemboli formation limit the launch of some studies [20]. Escaping these drawbacks could be achieved by using MSC secretome-derived products. However, despite the advantages of the MSC secretome over cell therapy, its application is also limited by the complexity of its standardization [21]. Notably, only a few number of studies aimed at exploring the pathogenic impact of a stem cell niche and the role of MSCs and their secretome in a stem cell niche recovery were carried out. Thus, to allow studying roles of MSCs and their secretome in SSC niche recovery, new animal models should be considered. Herein we focus on idiopathic male infertility modeling to expand the use of MSC-based products to address this unmet medical need.

Accumulating evidence points to the involvement of SSC niche components in the pathogenesis of idiopathic male infertility

It is estimated that infertility affects 8%–12% of couples worldwide, with the male factor being a primary or contributing cause in approximately 50% of couples. The increasing incidence of male infertility may be due to a multitude of factors, which can be stratified into congenital, acquired, and idiopathic factors. About 30%–50% of male infertility cases are idiopathic, with no discernible cause [22]. Men with a history of idiopathic infertility do not have obvious fertility problems, and physical examination and laboratory tests are normal. However, semen analysis detects sperm abnormalities that appear alone or in combination.

At present, only a number of possible causes and molecular mechanisms underlying idiopathic male infertility are known. In particular, genetic factors may be associated with male infertility. However, the majority of findings are so rare that additional independent cases are required to confirm the association of genetic factors with idiopathic male infertility as monogenic causes [23]. One can expect that next-generation sequencing (NGS)-based approaches might facilitate this task. Although NGS analysis of custom gene panels was able to identify genetic events possibly involved in idiopathic male infertility development [24,25], the majority of the cases remained unexplained. This suggests the possible involvement of other, non-genetic, factors in the pathogenesis of idiopathic male infertility.

Among the most studied epigenetic factors, global or gene-specific DNA methylation may be involved in the development of idiopathic male infertility, albeit the roles of histone modifications or chromatin protamination in spermatogenesis remain less evident [26]. Importantly, some signatures of differential DNA methylation regions may serve as predictors of response to particular treatments for idiopathic male infertility [27]. However, mechanisms leading to the alterations of DNA methylation profiles and their role in the development of male infertility still need elucidation. There have been extensive studies on other epigenetic factors such as miRNAs. However, similarly to previously discussed factors, additional data are needed to prove the presence of a relationship between aberrant miRNA expression and idiopathic male infertility [28].

Apparently, aberrant mRNA expression might be related to idiopathic male infertility. First, it has been shown that a bulk of the genes associated with idiopathic male infertility is related to reactive oxygen species (ROS). This could explain the significance of imbalance in ROS genes and their protein products present in seminal plasma as well as in the membrane components of damaged spermatozoa [29]. These findings are consistent with those of the overexpression of glutathione transferase genes in non-obstructive azoospermia(NOA) and oligospermia samples [30], where these genes were found to possibly detoxify ROS [31]. Second, aberrant expression can be observed not only in the cells of the spermatogenic epithelium, but also in somatic cells [32]. Accordingly, immature Sertoli cell fractions were revealed transcriptomes of single Sertoli cells obtained from patients with idiopathic male infertility. These groups exhibited enriched Gene Set Enrichment Analysis terms characteristic of infantile and pubertal Sertoli cells, were more proliferative in vitro, and had energy metabolism patterns typical to immature Sertoli cells. The ability of immature Sertoli cells to support germ cell colonies were worse, yet statistically insignificant, compared to normal adult Sertoli cells. Importantly, the functional immaturity of isolated Sertoli cells was curable by Wnt pathway inhibition that reveals the ability for therapeutic modulation of SSC niche properties [33]. Furthermore, some clinical studies showed that men with a history of idiopathic male infertility demonstrated significant signs of impaired Leydig cell function. That could be caused by disturbed paracrine communication between the seminiferous epithelium and Leydig cells or congenital dysfunction of both components [34]. Additionally, in a mouse model, the production of glial cell line-derived neurotrophic factor(GDNF) by peritubular myoid cells was demonstrated to be essential for spermatogonial development [35]. As testosterone induces GDNF expression, this might indicate the significance of the interplay between SSC niche components such as Leydig cells and peritubular myoid cells.

Taken together, many factors may be involved in the pathogenesis of idiopathic male infertility. Accumulating evidence points to the substantial role of SSC niche components in the pathogenesis of this disease.

MSCs and their secretome can recover injured stem cell niches

Multipotent stem and progenitor cells support the structure of tissues and their potency to renew and regenerate throughout the life. In order to do this, they require coherent regulation by the microenvironment, a stem cell niche that is made up of cells and cell–cell contacts, paracrine factors, metabolites, extracellular matrix (ECM), and physical and chemical cues. Given that a niche can regulate quiescence and differentiation of stem cells, the loss of individual components of a niche can affect its regenerative capacity [36–38]. Therefore, to be able to recover, a niche might exploit internal mechanisms preventing itself from degradation. Along with other niche components [39], MSCs might play a pivotal role in this process. Particularly, to replenish altered niche cellular components, MSCs might act as their precursors [40]. Nevertheless, at present, the importance of the paracrine effects of MSCs in restoring the cellular composition of tissue is generally accepted. Particularly, MSCs secrete cytokines and growth factors that protect tissue from damage by regulating the immune response, stimulating angiogenesis, and maintaining the viability of the microenvironment [11]. Paracrine secretion of MSCs also involves ECM components that may fulfill structural and signaling roles [41]. Extracellular vesicles secreted by MSCs can be important for tissue regeneration as well due to miRNA, mRNA, bioactive lipid, and protein transfer [42]. Moreover, MSC-derived apoptotic bodies may also be involved in tissue regeneration [43].

Cells that meet the minimal MSC characterization criteria have been found in many tissues of the body [16]. They are also present in many stem cell niches and can participate in the regeneration of injured stem cell niches as endogenous components [44,45]. This raises the question of the possibility of the effective use of MSCs isolated from a different tissue source in regenerative medicine [46]. Characterization and analysis of MSC secretome might shed light on this issue given that secretion of paracrine factors along with ECM components and extracellular vesicles by MSCs is accepted to be pivotal for tissue regeneration [47]. Indeed, the heterogeneity of MSCs is inherent among donors, cell populations from different tissues, or different phenotypes [48]. Furthermore, the secretome of MSCs demonstrates dissimilar functional properties [49] that might persist after transplantation to another tissue [50]. However, in some injury models, they overlap substantially and might be interchanged [51]. Furthermore, while administrating MSCs from a different tissue, the ability of the microenvironment to impose tissue-specific regenerative properties to transplanted cells might also limit heterogeneity and increase the efficacy of MSCs [41,52,53].

These hypotheses have been proven by distinct studies of MSCs or their secretome for the recovery of injured stem cell niches [54–56]. However, new models and indications are needed to make these efforts more effective and reproducible. In particular, SSC niche injury, as a model of idiopathic male infertility, can be used to prove the applicability of MSCs or MSCs secretome as an effective treatment for regeneration of an injured stem cell niche.

Application of MSCs or their secretome might be effective for the treatment of idiopathic male infertility

Indeed, a large number of studies are devoted to the use of MSCs as a "tool" for the restoration of spermatogenesis. In particular, it was demonstrated that the intratesticular injection of bone marrow MSCs promoted almost complete normalization of spermatogenesis in busulfan-treated hamsters [57]. Similar results have been shown in other animal models of busulfan-induced azoospermia [58]. For some models, it can be assumed that spermatogenesis might be restored due to paracrine stimulation provided by transplanted MSC to maintain the function of injured or dysregulated SSC niche components [59]. Moreover, the impact of paracrine factors secreted by MSCs on spermatogenesis restoration can be confirmed by the effective restoration of spermatogenesis in animal models of spermatogenesis failure after injection of human MSCs isolated from various tissues or their secretome [60–62].

Taken together, the application of MSCs or their secretome might be effective to resolve injury to spermatogenesis in various animal models. Therefore, the application of MSCs or their secretome for idiopathic male infertility treatment might be considered. To guarantee the best potency of MSC-based products, the model should reflect the possible role of the SSC niche in the pathogenesis of idiopathic male infertility.

Searching for a feasible toxicant to model SSC niche injury

Since idiopathic male infertility is a multifactorial disease, different SSC niche components should be damaged in a relevant idiopathic male infertility model. Additionally, the modeled injury to a niche should be, at least, partially reversible to allow therapeutic restoration of spermatogenesis. Among fully reversible models, one can name ischemic models [63]. However, in a reversible setting, spermatogenic epithelium is predominantly damaged conversely to somatic niche components. Apparently, as germ cells are more sensitive to ischemia than Sertoli cells and particularly Leydig cells, it seems impossible to damage SSC niche components while saving germ cell epithelium. Thus, testicular torsion for sufficient time periods to cause loss of all germinal elements in the testis still leaves the testis with considerable steroidogenic capacity [64]. Similarly, physically modeling SSC niche injury by testicular puncture might also be suboptimal as it can lead only to local disturbances [65]. Modeling of abdominal cryptorchidism seems to be a fairly optimal way to disrupt the SSC niche as it allows it to affect several components of the SSC niche. An additional advantage of this model is the reversibility of the modeled injuries [66]. However, to model

Table 1 Impact of chemicals on an SSC niche injury.

Chemical	SSC Toxicity	Sertoli cell toxicity	Leydig cell toxicity	Peritubular macrophage toxicity	Peritubular myoid cell toxicity	Reversible/ irreversible damage	References
Cisplatin	yes	no data	yes	no data	no data	Reversible	[85]
Doxorubicin	yes	yes	yes	no data	no data	Reversible	[81,86,87]
Cyclophosphamide	yes	–	–	no data	no data	Irreversible in combination (CHOP)	[81,88]
Chlorambucil	yes	–	yes	no data	no data	Reversible	[88,89]
Melphalan	yes	–	–	no data	no data	Reversible	[89,90]
Ifosfamide	yes	–	–	no data	no data	Reversible	[91]
Carmustine	yes	–	yes	no data	no data	Irreversible in combination (BEAM)	[92]
Busulfan	yes	yes	yes	no data	yes	Reversible	[59,93,94]
Cytarabine	yes	no data	–	no data	no data	no data	[80]
Vinblastine	yes	yes	no data	no data	no data	Reversible	[81,82]
Vincristine	yes	no data	yes	no data	no data	Irreversible in combination (BEACOPP)	[83]
Etoposide	yes	no data	no data	no data	no data	no data	[84]

Abbreviations: CHOP: combination of cyclophosphamide, doxorubicin hydrochloride (hydroxydaunorubicin), vincristine sulfate, and prednisone; BEAM: combination of carmustine, etoposide, cytarabine, and melphalan; BEACOPP: combination of bleomycin sulfate, etoposide phosphate, doxorubicin hydrochloride, cyclophosphamide, vincristine sulfate, procarbazine hydrochloride, and prednisone.

abdominal cryptorchidism, animals must undergo two surgeries, which is less ethically acceptable and might be accompanied by an increased risk of infections.

Consequently, toxicant- and radiation-induced models might be more appropriate. Among the most common chemicals able to injure SSC niche, ethane-1,2-dimethanesulfonate or mono- (2-ethylhexyl) phthalate act primarily on Leydig cells or Sertoli cells, respectively [67,68]. Conversely, the methods of radiation exposure are dangerous mainly for the spermatogenic epithelium [69]. Therefore, other toxicants for modeling SSC niche injury can be considered [70]. To select an optimal chemical to damage the SSC niche and subsequent disorders of spermatogenesis, their modes of action along with reversibility of their effects within SSC niche were analyzed.

As a result of a literature search, doxorubicin might apparently be used to model idiopathic male infertility [Table 1]. Doxorubicin has the potential to cause cell death or cell growth arrest by inhibiting topoisomerase II and DNA intercalation, and the ability to turn into free radicals, initiating ROS production and inducing oxidative damage to cellular DNA and the mitochondria. Currently, it is believed that doxorubicin-induced cell death predominantly involves the function and dysfunction of the mitochondria and cell energy levels, which may provide a low selectivity for doxorubicin [71]. At least by stimulating the production of ROS, doxorubicin may damage somatic components of the SSC niche; however, not limited to this [72,73], an excess of which is characteristic of idiopathic male infertility [29]. Another advantage of doxorubicin is its relatively wide therapeutic interval and the ability to model dose-dependent effects [74]. Concurrently, the damaging effects of doxorubicin can be reversible [75]. In summary, doxorubicin may be the toxicant of choice for modeling idiopathic male infertility.

It is reasonable to select a control substance that will make a significant contribution to the restoration of spermatogenesis in order to use the doxorubicin-induced injury model. Given the mechanism of action of doxorubicin, one might consider antioxidants. First, although more reliable confirmation of the effects is required through large-scale placebo-controlled studies, the results of several clinical trials suggest that antioxidants (vitamin E, zinc sulfate) may increase the chances of conception in patients with idiopathic male infertility [76,77]. Further, according to published results [78,79] and our unpublished data, antioxidants might serve as control treatments in regenerative medicine therapeutics studies on idiopathic male infertility (Fig. S1, unpublished data).

Conclusions

Many injury models have demonstrated that regenerative medicine products, particularly MSC-based, can mediate the restoration of damaged tissue by recovering a stem cell niche. It is also suggested to further consider MSCs and their secretome in the treatment of male infertility disorders associated with a SSC niche injury. Still, a deeper study of their mode of action might be required to enhance potency. Therefore, relevant animal models of the SSC niche injury are needed to

resolve this challenge. In this case, doxorubicin-induced injury might be promising due to its rather broad spectrum of toxicity and the reversibility of its damage. Importantly, features of this toxicant might be used in other stem cell niche injury models to study the potency of other novel drugs for regeneration.

Summary

In this review, the authors critically considered the promises and limitations of regenerative medicine to treating idiopathic male infertility focusing on the tight link of its pathogenesis with the SSC niche disturbance. The authors suggest that approaches aiming at launching the regeneration of an altered stem cell niche might be worth considering to overcome this disease entity.

In this regard, multipotent mesenchymal stromal cells (MSCs) could be proposed as a promising tool for the stimulation of the SSC niche restoration. However, the successful application of MSC-based treatment is limited by the uncovered mechanisms of action of MSCs and their secretome. Therefore, attention is paid to specific animal models that should be developed or adapted to reveal the role of MSCs and their secretome in a stem cell niche recovery. Hence, the authors conduct an in-depth comparative analysis of spermatogenesis disorder models in order to select an experimental exposure that damages the somatic components of the SSC niche. As a result, doxorubicin is suggested as a toxicant of choice for modeling the idiopathic male infertility, which is supported by our own experimental data.

Of note, many injury models have demonstrated that MSC-based products can mediate the restoration of damaged tissue by recovering a stem cell niche. In this case, use of doxorubicin-induced SSC niche injury might be successfully applied to other stem cell niche injury models to study the potency of other novel drugs for regeneration.

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Ethics declarations

Animals were housed and used for experimental procedures in full compliance with Directive 2010/63/EU. The work was approved by local ethic committee (#90-G).

Consent for publication

All authors have agreed to publish this manuscript. All materials and images are original. No consent needs to declare.

Conflicts of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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Appendix A. Supplementary data

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