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Review Article

MiRNA regulated therapeutic potential of the stromal vascular fraction: Current clinical applications - A systematic review

Murad Agaverdiev [a], Bedil Shamsov [a], Sorbon Mirzoev [b], Andranik Vardikyan [a], Manuel Encarnacion Ramirez [c], Renat Nurmukhametov [d], Aferin Beilerli [f], Bohan Zhang [e], Ilgiz Gareev [g,*], Valentin Pavlov [a]

[a] Department of Urology, Bashkir State Medical University, 450008, Ufa, Russian Federation
[b] Department of Urology, City Clinical Hospital, №21, 450071, Ufa, Russian Federation
[c] Department of Neurosurgery, Peoples' Friendship University of Russia (RUDN University), 6 Miklukho-Maklaya Street, Moscow, 117198, Russian Federation
[d] Division of Spine Surgery, Central Clinical Hospital of the Russian Academy of Sciences, Moscow, Russian Federation
[e] Department of Neurosurgery, The First Affiliated Hospital of Harbin Medical University, No. 23, Youzheng Street, Harbin, 150001, China
[f] Department of Obstetrics and Gynecology, Tyumen State Medical University, 54 Odesskaya Street, 625023, Tyumen, Russia
[g] Central Research Laboratory, Bashkir State Medical University, 450008, Ufa, Russian Federation

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ABSTRACT

Introduction: The stromal vascular fraction (SVF) is a heterogeneous population of cells that, interacting with each other, can affect the processes of regeneration, angiogenesis, and immunomodulation. Over the past 20 years, there has been a trend towards an increase in the number of clinical studies on the therapeutic use of SVF. MicroRNAs (miRNAs) are also important regulators of cellular function and they have been shown to be involved in SVF cellular component function. The purpose of this study was to analyze existing clinical studies on the therapeutic use of SVF including the role of miRNAs in the regulation of the function of the cellular component of SVF as an anti-inflammatory, pro-angiogenic and cell differentiation activity.

Methods: The search strategy was to use material from the clinicaltrials.gov website, which focused on the key term "Stromal vascular fraction", and the inclusion and exclusion criteria were divided into two stages.

Results: By August 2022, there were 149 registered clinical trials. Most studies belong to either Phase 1–2 (49.37%), Phase 1 (25.32%) or Phase 2 (22.78%). Most of them focused in the fields of traumatology, neurology/neurosurgery, endocrinology, vascular surgery, and immunology. However, only 8 clinical trials had published results. All of clinical trials have similar preparation methods and 8 clinical trials have positive results with no serious adverse effects

Conclusions: There appears to be a wide potential for the clinical use of SVF without reports of serious side effects. Many preclinical and clinical studies are currently underway on the use of SVF, and their future results will help to further explore their therapeutic potential. Nevertheless, there are not many studies on the role of miRNAs in the SVF microenvironment; however, this topic is very important for further study of the clinical application of SVF, including safety, in various human diseases.

1. Introduction

The stromal vascular fraction (SVF) is a stromal tissue that contains many different stem cells, as well as other supporting cells and signaling molecules. This cell mixture, traditionally isolated by enzymatic treatment, contains several cell populations including mesenchymal stem cells (MSCs), endothelial progenitor cells (EPCs), immune cells, vascular smooth muscle cells (VSMCs), pericytes and other stromal components [1]. This unique set of cells facilitates a number of biological processes, including accelerated healing, reduced inflammation, angiogenesis, immune modulation, and a range of local and systemic effects mediated by cytokines [1,2]. Although some studies have used a homogeneous adipose-derived cell population to enhance stromal cell proliferation/angiogenesis, it is important to understand that SVF is a complex cellular system that has clinically relevant potential in therapy, and not just a homogeneous cell type (Table 1) [3].

* Corresponding author.
E-mail address: ilgiz_gareev@mail.ru (I. Gareev).

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Table 1
The most important cellular component of stromal vascular fraction (SVF) and their respective surface markers.

Cell population of SVF	CDs and other markers	
	Positive	Negative
Adipose tissue-derived stem cells	CD13, CD90, CD73, CD34, and CD29	CD45, CD144 and CD31
Human endothelial progenitor cells	CD133, CD146, CD31, and CD34	CD45
Endothelial cells	FVIII, CD31	CD34
T-lymphocytes	Foxp3, CD8, CD4, and CD25	–
Macrophages	CD45, CD14, CD34, and CD206	–
Smooth muscle cells	Alpha-actin	–
Pericytes	CD73, CD44, CD29, CD13, CD146, and CD90	CD45, CD34 and CD56
Preadipocytes	CD34	CD45, CD31 and CD146

The regenerative properties of SVF are explained by its paracrine effects. SVF cells secrete certain factors - vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and transforming growth factor-β (TGF-β) in the presence of various stimuli such as hypoxia, affecting stem cells differentiation, angiogenesis, and wound healing, and potentially promoting the growth and development of new tissues [4,5]. Due to these properties and ease of cell harvesting with minimal damage to the donor area, SVF is especially promising for regenerative medicine. *In vitro* studies quickly evolved into *in vivo* experiments testing SVF to evaluate their ability to effectively regenerate and repair tissues or organs [6–8].

MicroRNAs (miRNAs) are short, on average 18–20 nucleotides (nt), single-stranded non-coding RNAs that regulate gene expression at the post-transcriptional level by binding to the 3′-untranslated region (3′-UTR) of specific target mRNAs, which leads to a decrease in protein expression through blockade translation and (or) contributing to the degradation of mRNA targets [9]. MiRNAs are involved in almost all biological processes, including cell proliferation, apoptosis, and cell differentiation. It is knowing that the adipogenic, chondrogenic and osteogenic differentiation of SVF cells is regulated by various factors including miRNAs [10,11]. For instance, Pan et al. in their study characterized the role and mechanism of miR-124-3p in the adipogenic differentiation of SVF isolated from sheep subcutaneous fat [12]. Their study showed that miP-124-3p inhibited SVF lipid droplet formation by targeting enhancer-binding protein alpha (C/EBPa), a transcriptional regulator of adipogenesis. These results facilitate understanding of the mechanisms of development and metabolism of subcutaneous adipose tissue at the post-transcriptional level. In other study, Zhao et al. demonstrated that miR-301a attenuates the adipogenic differentiation of ovine SVFs by targeting homeobox C8 (HOXC8), where known that HOXC8 involved in lipid homeostasis [13]. The results of these studies show the direct role of post-transcriptional regulation, represented by miRNAs, of SVF function.

By August 2022, about 149 registered SVF-clinical trials at clinicaltrials.gov use SVF as therapeutic agents. Unfortunately, from these studies, only 8 have published results and results of 15 studies were withdrawn. The objective of this systematic review is to analyze the different applications of SVF in a clinical setting to illustrate the growing and broad potential of their therapeutic applications that could aid in the reconstruction of damaged tissues and potentially save many lives. Also, we focus on the relevant isolation and treatment methods, and as well as their storage conditions.

2. Material and methods

2.1. Search strategy

Clinicaltirals.gov and PubMed was chosen because it includes most of the current clinical trials worldwide and has downloadable data for statistical analysis. The “miRNAs” and “Stromal-vascular fraction” was chosen by the selected keywords. In this systematic review, the following inclusion and exclusion criteria were used to select studies and where it was divided into two stages:

Stage 1:

1) Human clinical trials using SVF; 2) Clinical trials at Phase 1 to 4: a. - location, b. - field of application, c. - Phase, and d. - status.

Stage 2:

1) Clinical trials that have published outcome results; 2) Phase 2 with the selected clinical trial criteria, that allows a statistical analysis: a) field of application, b) treatment, c) intervention model, d) source, e) preparation methods, and f) results.

2.2. Clinical trials selection

After applying the inclusion and exclusion criteria, for stage 1, a total of 69 clinical trials were removed because they were either at early Phase 1, phase not specified or had an inapplicable phase (Note: except for those who have results). From the remaining 80, we analyzed their field of application, Phase, status, and location. For stage 2, only 8 clinical trials were selected because they fit the stated criteria (Table 2).

3. Results

The therapeutic application of SVF is a relatively new area, but in terms of indications, it has great potential for solving many serious health problems in a large number of fields of clinical medicine (Fig. 1). More research may be needed to exploit their full therapeutic potential, but it is safe to say that significant progress has been made in exploring the usefulness of using SVF for regenerative and immunomodulatory treatments.

3.1. Findings from clinical trials

Progress by August 2022, there were 149 registered clinical trials worldwide using SVF to investigate their therapeutic potential. There has been a significant increase in registered clinical trials at clinicaltrials.gov since the first one was reported in June 23, 2008. 13 started between 2008 and 2012, 77 were initiated between 2013 and 2017, and 59 began between 2018 and 2022 (Fig. 1).

In total, 69 clinical trials were removed following the inclusion and exclusion criteria: 4 were on an early Phase 1, 13 clinical trials with Phase not specified and 52 had a not applicable phase. Nevertheless, with the inclusion and exclusion criteria, 80 were analyzed. Of the remaining clinical trials, 21 clinical trials were in only phase 1, 18 in only Phase 2, 39 in Phase 1–2, 1 in Phase 3, and 1 in Phase 4. However, from this not large amount of clinical trials, only 8 have published the outcomes. Regarding the status of the remaining clinical trials, 11 have not started to recruit, 26 were recruiting, 7 were enrolling by invitation, 5 were active but not recruiting yet, 3 were suspended, 6 were terminated, 41 were completed, 15 had withdrawn, and 35 have an unknown status (Figs. 2 and 3).

3.2. Geographic distribution

The currently selected 80 clinical trials are located in 32 countries around the world. The United States and France are leading the research with 66 and 11 clinical trials, respectively. They are followed by China with 8, Honduras 5, Russian Federation 7, Denmark 4, Republic of Korea 4, and India 4 (from all 149 clinical trials which registered on clinicaltrials.gov) (for descriptive reasons only the top 8 countries were included, for the complete description Fig. 4). Many of these clinical

Table 2
Distribution of the highest performing clinical trials (with results) registered on chttp://linicaltrial.gov by the use stromal vascular fraction (SVF).

Conditions and diseases	Number of participants	Interventions	Study Type	NCT Number	Phase	Brief Summary	Ref.
Breast reconstruction, contour irregularities and volume insufficiency	20	Centrifuged fat graft; ADSCs enriched fat graft	Interventional	NCT01771913	Phase 2	The purpose of this study is to investigate if there is a relationship between the take of fat grafts with and without adipose-derived stromal cells (ADSCs) and the presence of specific surface markers on the cells of the stromal vascular fraction	[14, 15, 16, 17]
Systemic sclerosis	20	SVF injection	Interventional	NCT03060551	Early Phase 1	This study outlines the safety of the autologous SVF cells injection in the hands of patients with systemic sclerosis. Preliminary assessments at 6 months will suggest potential efficacy needing confirmation in a randomized placebo-controlled trial on a larger population	[18]
Osteoarthritis	39	GID SVF-2; Placebo	Interventional	NCT02726945	Not Applicable	This is a pivotal study. The study will examine the safety and efficacy of autologous SVF cells processed with the GID SVF-2 device for pain, function and stiffness in the knees of osteoarthritic subjects.	[19, 20, 21]
Androgenetic and alopecia	7	GID SVF-2	Interventional	NCT02626780	Not Applicable	The general objective of this study is to conduct a safety and feasibility study of a single injection of autologous adipose-derived SVF for the treatment of alopecia.	*
Wounded warrior, limb shortening and amputation	10	Enhanced fat grafting; standard fat graft	Interventional	NCT02076022	Not Applicable	We propose a prospective, randomized clinical study to assess the efficacy of minimally invasive autologous fat transfer addressing pain and poor prosthetic fit at amputation sites.	*
Osteoarthritis of the knee	6	ADSC	Interventional	NCT02357485	Phase 1	This safety and feasibility study used autologous ADSC, the SVF, to treat 8 osteoarthritic knees in 6 patients of grade I to III (K-L scale) with initial pain of 4 or greater on a 10-point scale, under Institutional Review Board (IRB) approved protocol.	*
Facial injuries	15	Fat grafting; general anesthesia; coleman cannulas; tefla non-adherent gauze pad	Interventional	NCT02267187	Not Applicable	This study will examine the impact of the fat grafting procedure on facial appearance and quality of life over time by precisely measuring soft tissue volume with CT scans, assessing appearance with 2D and 3D photography and standard photography and evaluating quality of life through various validated psychosocial measures. The study endpoints include the analysis of the graft site via study procedures at different time points, the comparison of cotton rolling to centrifugation method of autologous fat grafting, as well as the correlation of cell behavior of the laboratory assays with clinical outcomes.	[22]
Craniofacial injuries	5	Fat grafting	Interventional	NCT01633892	Not Applicable	This study is the second of two clinical studies at the University of Pittsburgh using each person's fat graft with concentration of fat cells in the graft to observe if there is less fat resorption compared to using fat grafts alone. Each study is using a different concentration of fat in the fat graft compared to the first clinical study.	[17, 23, 24, 25, 26, 27, 28]

Note: Includes publications given by the data provider as well as publications identified by ClinicalTrials.gov Identifier (NCT Number) in Medline.

trials (80 clinical trials) are located in different countries, highlighting the importance of international research collaboration. Of the 20 clinical trials in only Phase 1: 12 are located in the United States, 1 in China, 3 in Russian Federation, 1 in Denmark, 2 in Honduras and 1 in Canada. Of the 16 clinical trials in only phase 2: 5 are located in the United States, 4 in France, 2 in Uganda, 1 clinical trial each from the following listed countries as Serbia, Switzerland, Mexico, Brazil, and Pakistan. Of the 42 clinical trials in phase 1–2: 10 are located in the United States, 5 in China, 4 in India, 4 in Russian Federation, 2 clinical trials each from the following listed countries as Vietnam, Honduras, France, Poland, France, Spain, Philippines, Panama and 1 clinical trial each from the following listed countries as Nicaragua, Denmark, Iran, Turkey, and

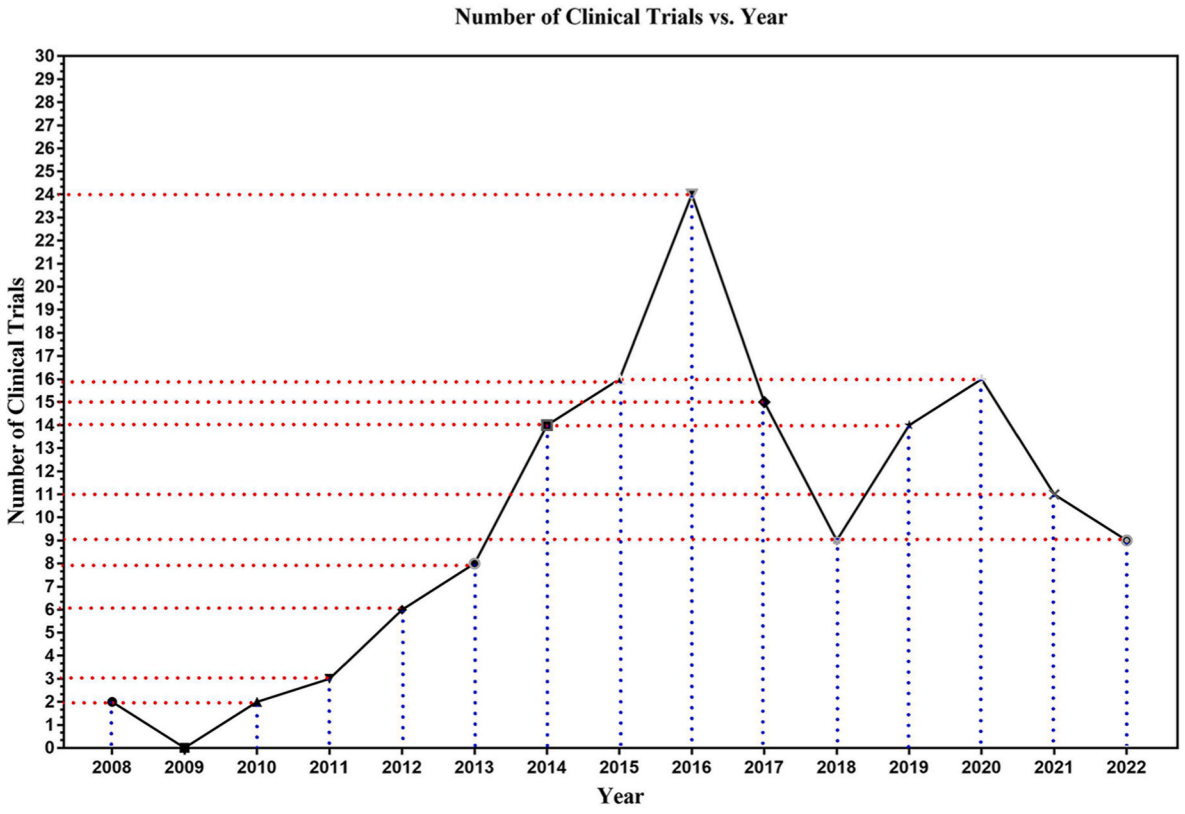

Fig. 1. Statistics of registered clinical trials for testing the clinical potential of the stromal vascular fraction (SVF) in accordance with the dynamics of temporary (year) registrations since the start of the first clinical trial.

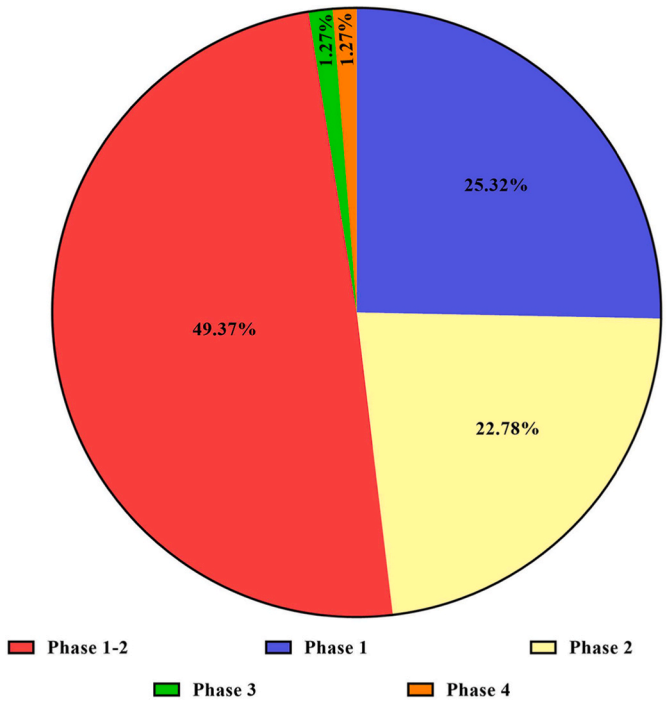

Fig. 2. Distribution of registered clinical trials on the use of stromal vascular fraction (SVF) by phases.

Bangladesh. Finally, of the 2 clinical trials in Phase 3 and Phase 4: 1 is located in the United States and 1 in Poland (for the complete description Table 3).

After the United States and France that have the highest number of registered clinical trials, a comparison of the other countries conducting clinical trials show a greater percentage of their clinical trials already in Phase 2: Uganda 2 (12.5% from 16 clinical trials), Serbia, Switzerland, Mexico, Brazil, and Pakistan 5 (31.25% from 16 clinical trials), whereas France and the United States 9 (56.25% from 16 clinical trials). Interestingly, of the clinical trials already in Phase 3 and Phase 4, only two countries, the United States and Poland, with 2 clinical tials (50.0% and 50.0% from 2 clinical trials, respectively).

3.3. Findings from methodologies employed to supply SVF for clinical trials

Most clinical studies have detailed information on SVF isolation procedures used in their protocols. In all cases, autologous adipose tissue was used. After extracting SVFs from their natural source, they went through various stages of purification and expansion for use in patients. Typically, these procedures include isolation, expansion (optional), the used of different kits to extract certain subpopulations of cells from SVF (e.g., adipose tissue-derived stem cells (ADSCs)), harvested and cryopreservation. All of these methods were performed in certified prep laboratories in accordance with Good Manufacturing Practice (GMP).

In addition, SVF subpopulations were characterized by flow cytometry to confirm their identity and purity. Of the selected studies with phases indicated, the medical specialties with the highest number of studies reported were traumatology, neurology, vascular surgery, plastic surgery, rheumatology, and neurosurgery. The most common pathologies were osteoarthritis, atherosclerotic vascular lesions (e.g., ischemia of the lower extremities), diabetes mellitus and its complications, and inflammatory bowel disease. All of these clinical studies were either in Phase 1 or Phase 2. The number of cellular subpopulations of SVF used varied where highlighting the importance of cell expansion. Clinical studies with results have shown positive results without serious side

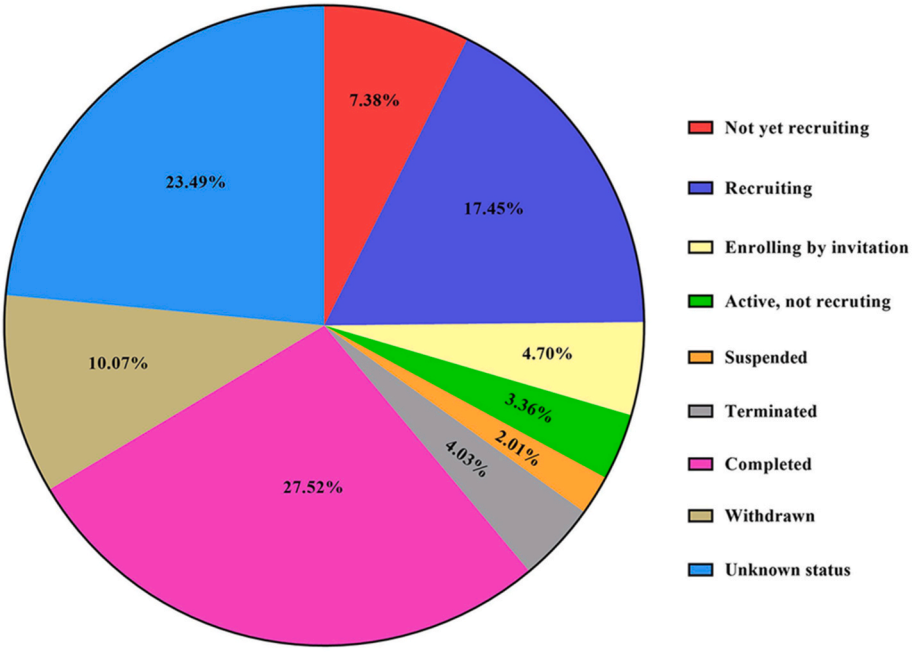

Fig. 3. Distribution of registered clinical trials on the use of stromal vascular fraction (SVF) depending on progress.

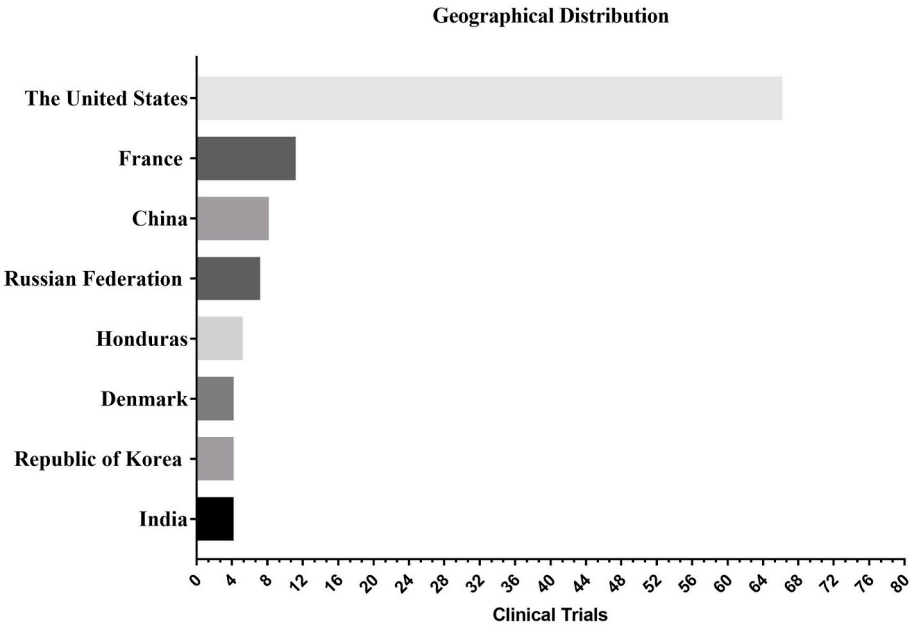

Fig. 4. Distribution of registered clinical trials on the use of stromal vascular fraction (SVF) by localization in the world.

effects. A more detailed description of their procedures and results can be found in Table 1.

3.4. MiRNAs and SVF microenvironment

The main mechanism of action is based on a complex relationship between a heterogeneous SVF cell population and recipient cells, leading to stimulation of cell differentiation, angiogenesis, and immunomodulatory/anti-inflammatory, and, accordingly, restoration of damaged cells and tissues [14,15].

The immunomodulatory effect of SVF is determined by the presence of ADSCs and a population of immune cells [16]. The mechanism of their action in this case can be based both on homing and differentiation into site-specific differentiated cells, stimulation of tissue stem cells of the recipient, and on the paracrine effect. Moreover, the paracrine effect of ADSCs can be provided both by direct interaction with recipient stem cells and cells of the immune system (cell–cell contact) and by soluble factors [17–19]. ADSCs produce a huge number of growth factors and cytokines with immunosuppressive, antiapoptotic, antifibrotic, and angiogenic effects. The ability of SVF to stimulate angiogenesis has been proven in many studies, which is of particular importance in the treatment of conditions accompanied by ischemia and decreased vascularization [20–22]. In addition to VEGF, angiogenesis factors produced by SVF cells such as fibroblast growth factor-β (FGF-β), hepatocyte growth factor (HGF), platelet-derived growth factor subunit B (PDGFB), and transforming growth factor-β (TGF-β) are also involved in this process

Table 3
Listed stromal vascular fraction (SVF) clinical trials with around the world.

Country	Total	Percentage	Phase 1	Phase 2	Phase 1-2	Phase 3	Phase 4
Uganda	2	2.5%		2			
Honduras	4	5.0%	2		2		
Nicaragua	1	1.25%			1		
Panama	2	2.5%			2		
China	6	7.5%	1		5		
Denmark	2	2.5%	1		1		
France	6	7.5%		4	2		
Poland	3	3.75%			2		1
Serbia	1	1.25%		1			
Spain	2	2.5%			2		
Switzerland	1	1.25%		1			
Iran	1	1.25%			1		
Turkey	1	1.25%			1		
Canada	1	1.25%	1				
Mexico	1	1.25%		1			
United States	28	35.0%	12	5	10	1	
Russian federation	7	8.75%	3		4		
Brazil	1	1.25%		1			
Bangladesh	1	1.25%			1		
India	4	5.0%			4		
Pakistan	1	1.25%		1			
Philippines	2	2.5%			2		
Vietnam	2	2.5%			2		
Total	80	100%	20	16	42	1	1

[23–26]. In addition, macrophages derived from the isolation of SVF from adipose tissue have an anti-inflammatory phenotype (M2) and may play a role in reducing inflammation [27,28].

The mechanisms underlying the regulation of proliferation or multipotency of ADSCs, another cellular component of SVF, and their synthesized factors via specific miRNAs and/or their target genes, have not been thoroughly investigated and therefore not well characterized. We hypothesized that the expression of some miRNAs in cultured SVF cells may partially explain the loss of therapeutic efficacy, and even affect safety. We also suggested that the knowledge of these changes can be used as a new opportunity to control the fate and activity of the SVF cellular component. Fig. 5 clearly shows the role of certain miRNAs on the function of the cellular component of SVF.

3.5. Exosomal miRNAs from ADSCs

Cell therapy never ceases to be popular in the field of medical sciences, especially in the field of regenerative medicine. Recently, adipose tissue has become the object of interest of many researchers due to the fact that it represents a new and potential source of cells for the purposes of tissue engineering and regenerative medicine. It is known that SVF carries a large number of stem cells and progenitor cells capable of self-

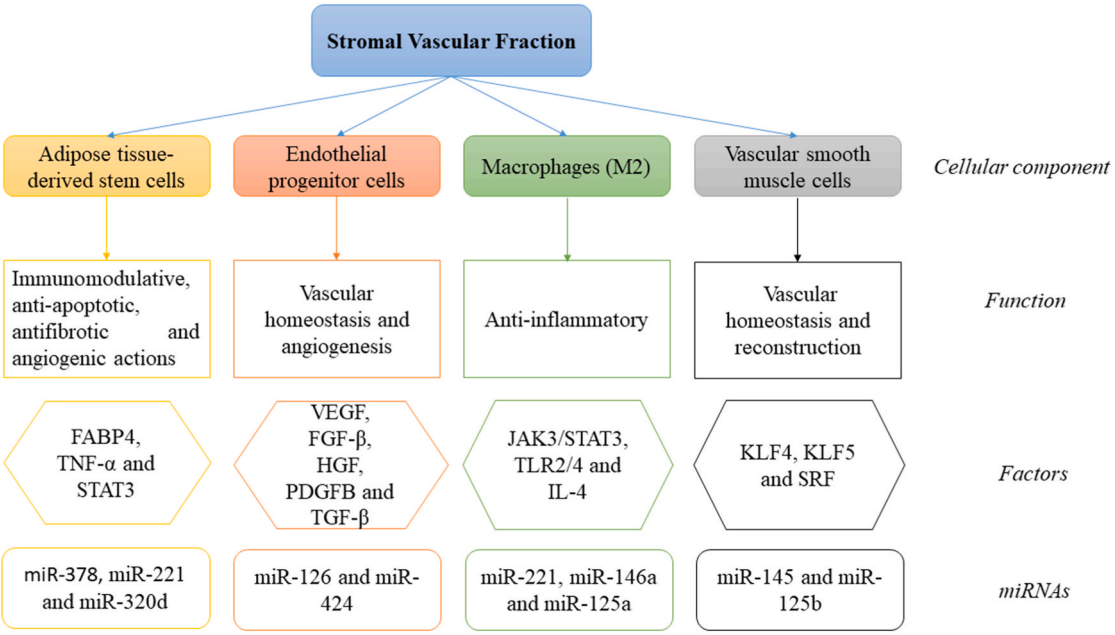

Fig. 5. Understanding stromal vascular fraction (SVF) cell biology by regulating miRNAs specific for cells component of SVF that regulate gene expression by targeting transcription factors associated to different processes. Note: FABP4, fatty acid binding protein 4; TNF-α, tumor necrosis factor-α; STAT3, signal transducer and activator of transcription 3; VEGF, vascular endothelial growth factor; FGF-β, fibroblast growth factor-β; HGF, hepatocyte growth factor; PDGFB, platelet-derived growth factor subunit B; TGF-β, transforming growth factor-β; JAK3, tyrosine-protein kinase; TLR2/3, toll-like receptor 2/3; IL-4, interleukin-4; KLF4/5, kruppel-like factor 4/5; SRF, serum response factor.

renewal, differentiation, and proliferation [20,23]. The development of strategies for regenerative medicine and tissue engineering or other area medicine, including the use of ADSCs from autologous adipose tissue, has particular promise for overcoming the disadvantages associated with the use of stem cells from other areas of the patient's body (for example, stem cells from the bone marrow) and finding solutions in the treatment of various human diseases [29–31]. ADSCs from SVF, like all stem cells, are able to detect and interpret biosignal signatures from damaged or degenerated tissue. For instance, ADSCs are in constant communication through biological signaling with the surrounding cellular environment, as well as through feedback signaling on their own cell membranes. This signaling system allows ADSCs to induce healing, stimulate angiogenesis, effectively maintain immunomodulatory effects and stop the escalation of pro-inflammatory status in vascular and musculoskeletal diseases, and act on damaged target cells from afar without actual engraftment in each case. Extracellular vesicles (EVs), in particular exosomes, which may contain miRNAs, can mediate this cell-to-cell communication [32]. As a result of the studied literature on the use of exosomal miRNAs obtained from ADSCs, several areas of medicine were identified in their potential clinical use, namely vascular diseases, diseases of the musculoskeletal system, oncology (in order to address the issue of tumor resistance to therapy), immunology, etc. [32–42]. This data was presented in the Fig. 6.

4. Discussion

SVF research has made substantial progress in the last two decades since they were first named. This study was focused on the therapy application of SVF, and clinical trials around the world with a focus on their clinical applications. Most were found being tested in traumatology and plastic surgery area and conditions like osteoarthritis, arthritis and elimination of cosmetic defects after removal of tumors (e.g., after a mastectomy); but there is also a significant number of clinical trials in the fields of neurology/neurosurgery, endocrinology, vascular surgery and immunomodulation of immune diseases such as host versus rheumatoid arthritis, systemic sclerosis and Crohn's disease. These clinical trials are pioneering a new frontier of innovative cellular therapies that offer potential benefits for the future of global public health as they target diseases for which there are currently no effective treatments, such as Crohn's disease, when both SVF and its cellular component showed initial positive results.

The growing number of clinical studies on the use of SVF represents a transition from studying a cultured and homogeneous population of cells to a heterogeneous mixture of SVF cells. Clinical trials, registered on clinicaltrials.gov, are devoted to the use of a heterogeneous population of SVF cells in the treatment of various human diseases, such as diseases of the musculoskeletal system. Ongoing clinical trials, most of which are in Phase 1 or Phase 2, seek to demonstrate the safety and efficacy of SVF therapy in humans. Given the promising results of past clinical studies on the use of SVF in therapy for various pathologies, it proves to us that SVF has great potential for use as a cellular therapy in humans.

As already known, adipose tissue is the largest reservoir of MSCs in the body and provides high-quality production of stromal cells [43]. Obtaining ADSCs from SVF is much easier and cheaper than any other type of MSC production [44]. Ready fresh autologous stromal cells are obtained right at the patient's bedside in a very short time. This feature is very important in the treatment of diseases of the immune system, since freshly thawed samples of MSCs are almost universally used in clinical trials; however, cryopreservation appears to impair the immunosuppressive properties of MSCs and shorten their *in vivo* lifetime [45,46]. When transplanted into damaged areas, SVF cells can interact with their neighboring microenvironment, which leads to the formation of new committed precursor cells. They secrete exosomes containing growth factors such as VEGF, cytokines, chemokines, and miRNAs involved in the repair of tissue defects and various biological functions [47]. These biomolecules play a critical role by stimulating the molecular mechanisms involved in angiogenesis, immunomodulation, and cell proliferation/differentiation, whereby repair of damaged tissue occurs [48,49].

The equipment, physician experience, and supplies required for the traditional method of isolating SVF are not common in most medical settings. Plastic surgery, which occupies the upper limit of medical expenses, is the largest consumer of SVF and related products, but the actual scope of its application is much wider. To date, there are automated biomedical devices that can produce injectable SVF from lipoaspirate. Such developments to create such equipment have been going on for quite some time, although mostly still in the testing phase, with Cytori's Celution® System (Cytori Therapeutics, San Diego, USA) being the first system. About 30 different automated and semi-automated systems are currently under development. The technologies and methodologies used vary, with most preferring the tried and tested enzymatic digestion method. Stempeutics Research Pvt. Ltd. (Bangalore, India) has developed one system such as Stempeutron™, evidence of positive results reported by SundarRaj et al. water fraction for isolation and concentration of SVF. The Stempeutron™ Filtration System is capable of capturing most of the therapeutically important cell types based on their size. Future developments with these systems will help make it possible to obtain specific cell populations that target specific diseases.

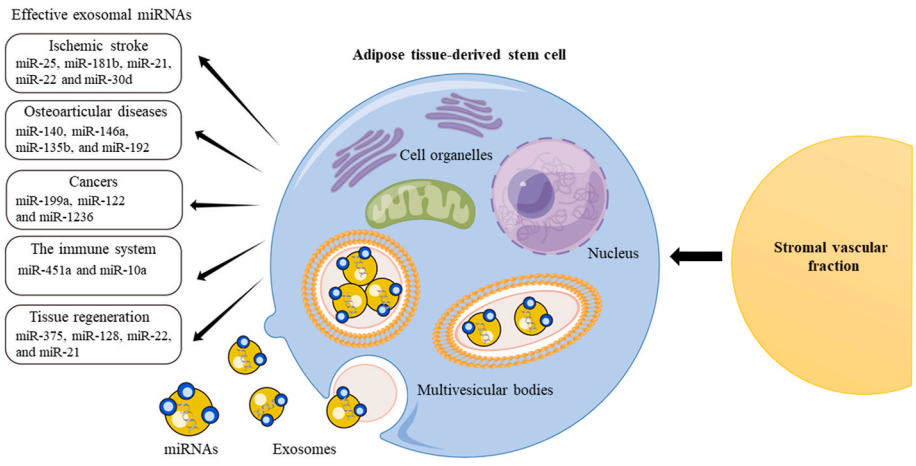

Fig. 6. Adipose tissue-derived stem cells (ADSCs) release exosomes with a miRNAs cargo by targeting transcription factors associated to different human diseases. These exosomal miRNAs can be used to develop new and effective therapies.

5. Limitations in the research process and author's opinion

At present, the clinical application of SVF/ADSCs is actively developing, and data on the therapeutic properties of these cell types are accumulating. SVF/ADSCs therapy is being studied in musculoskeletal disorders, complex neurological, immunological, cardiovascular and respiratory diseases. Encouraging results also demonstrate the study of the molecular processes of action of SVF and its cellular component, namely the role of miRNAs and downstream signaling pathways, followed by the study of the therapeutic potential of certain miRNAs. When using SVF/ADSCs in the treatment of severe, previously incurable human diseases, certain problems arise - scientific, ethical and legal. As already noted, the mechanisms that affect the efficiency of transplanted SVF, taking into account the "multicomponent" cellular composition, are not yet fully known; therefore, the consequences of using SPV for therapeutic purposes are often difficult to predict. It is known that the differentiation of ADSCs especially is influenced by numerous factors, such as mechanical tension, the volume and shape of the space occupied, electric fields, trophic factors, and cellular microcirculation. Unfortunately, it is not yet possible to ensure complete correspondence between the conditions of cell differentiation *in vivo* and in vitro, considering studies on the regulatory role of miRNAs. In addition, sometimes it is difficult to determine what exactly happened in the process of development of ADSCs - a fully functional specialized cell of the expected tissue, or, more often, an "intermediate" cell that carries on its surface several receptors characteristic of this type of tissue, but unable to completely replace defective cells. The second serious question, to which there is no unequivocal answer, yet, concerns the reaction of the obtained cells to the medicinal substances used by the patient. These questions raise a serious problem of assessing the quality of cells formed in the body.

The development of technologies for the use of SC is largely hampered by the ethical problems facing society. In the ethical review of any study, the risk/benefit ratio to which the subject is exposed is always assessed. The basic rule, the principle of biomedical ethics is that the interests of the patient (individual), along with the interests of society and the species prevail over the interests of science.

Since SVF/ADSCs have been used for a relatively short time, there have not yet been large-scale epidemiological and economic studies in this area. Especially according to clinicaltrials.gov, there is a downward trend in clinical trials, in particular in recent years. As a consequence, both regulatory and economic constraints due to the COVID-19 pandemic and the current adverse economic situation in the world are becoming barriers to wider clinical research and implementation of the use of SVF.

Further development of this area of medicine requires: 1) improvement of the legislative framework; 2) ccreation of cell banks equipped according to GTP rules; 3).training of specialists and improvement of the material base of clinics, since the proper production, preservation and use of individual SVF cells as ADSCs is one of the most complex technological processes; 4.development of clear indications and contraindications for the use of SVF/ADSCs.

In conclusion, we note that the use of SVF/ADSCs, despite the existing difficulties, is recognized by most experts as one of the most promising areas in the development of medicine in the 21st century. In our opinion, the intensity of ethical discussions will decrease with the achievement of more visible, large-scale and obvious results of the use of SVF in specific patients who previously had no hopes for a cure by the methods that are dominant in today's clinical practice.

6. Conclusion

To this day, the mechanism of the therapeutic effect of SVF is poorly understood and may depend on the state of the tissue/organ. SVF may well act in a variety of ways, and its biological activity may be determined by the microenvironment of the host tissue. In any case, given the complexities and unknown factors, creating activity assays to confirm accurate readings is challenging. The main actions of SVF are proangiogenic, antiapoptotic, antifibrotic, immunoregulatory, anti-inflammatory and trophic. Some of these actions may be associated with the presence of ADSCs (2–10% of the total SVF cell population). It is known that such morphological changes in organs or tissues as sclerosis develops as a result of impaired microcirculation and the development of hypoxia. In addition, inflammatory reactions are often accompanied by hypoxia, which as a result leads to active sclerosis of tissues or organs. This again confirms the potential role of SVF in the treatment of diseases accompanied by impaired trophism and blood circulation. There appears to be a wide potential for clinical use of SVF without serious side effects. However, there is still a long way to go before SVF can be introduced into routine clinical practice. There are currently 149 clinical trials listed on clinicaltrials.gov, but this number is expected to continue to grow. Increasing the number of clinical trials investigating the use of SVF in various human diseases will help determine the therapeutic potential of this source of cells and signaling molecules.

At the moment, it has been proven that miRNAs are involved in many processes of biological metabolism, including cell proliferation, cell differentiation, apoptosis, etc. More importantly, miRNAs also regulate cellular functions and the factors that they can synthesize. Taken together, the results of several studies suggest that the cellular component of SVF can effectively improve tissue healing and repair, have immunomodulatory and anti-inflammatory effects, and neovascularization by regulating specific miRNAs. All this confirms the complex molecular relationship between the cellular component of SVF and epigenetic regulation. Nevertheless, a more in-depth study of the role of miRNAs in the function of the cellular component of SVF will provide a clearer understanding of the therapeutic efficacy and safety of SVF.

Author contributions

Murad Agaverdiev and Ilgiz Gareev: conceptualization, project administration and writing – original draft. Andranik Vardikyan, Bedil Shamsov and Sorbon Mirzoev: writing – review and editing, investigation and resources. Manuel Encarnacion Ramirez and Renat Nurmukhametov: formal analysis and methodology. Bohan Zhang and Aferin Beilerli: data curation. Valentin Pavlov: validation, visualization, and funding acquisition. Valentin Pavlov: Supervision. All authors have read and agreed to the published version of the manuscript.

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Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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