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Review article

Myogenic stem cells

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Abstract: Both skeletal muscle and bone marrow tissue contain myogenic stem cells. The population residing in muscles is heterogenic. Predominant in number are "typical" satellite cells – muscle progenitors migrating from somites during embryonic life. Another population is group of multipotent muscle stem cells which, at least in part, are derived from bone marrow. These cells are tracked by gradient of growth factors releasing from muscle during injury or exercise. Recruited bone marrow-derived cells gradually change their phenotype becoming muscle stem cells and eventually can attain satellite cell position and express Pax7 protein. Mesenchymal stem cells (MSC) isolated directly from bone marrow also display myogenic potential, although methods of induction of myogenic differentiation *in vitro* have not been optimized yet. Concerning efforts of exploiting myogenic stem cells in cell-mediated therapies it is important to understand the cause of impaired regenerative potential of aged muscle. Up to now, most of research data suggest that majority of age related changes in skeletal muscles are reversible, thus depending on extrinsic factors. However, irreversible intrinsic features of muscle stem cells are also taken into consideration.

Keywords: satellite cells, stem cells, mesenchymal cells, skeletal muscle, bone marrow, ageing

Introduction

The most basic definition of stem cells says, that they are cells with the capacity to self renew and to differentiate into specialized cells. This definition, thanks to its simplicity, is still valid despite of constant updates in this field. There are different types of stem cells. The most primitive is totipotent zygote and its immediate progeny – primordial cells. They can give rise to all tissue and organs including placenta, but this is a transient population without ability to self-maintain and that is why it is called prestem cells. At the stage of blastocyst cells originating from inner cells mass are termed embryonic stem cells (ESC). They are pluripotent, which means the capability to differentiate into all three germ layers, but not into placenta any more (this is a unique feature of trophoblast stem cells). Further development cause the appearance of cells with a confined potency to differentiate into one of germ layer. They are called multipotent stem cells and can give rise to either endoderm, mesoderm or ectoderm [1,2]. With time of growing, the ability to multilineage differentiation gradually decreases and most of cells are directed onto certain path of development. Initially, stem cells were associated only with the prenatal stage, but later they were found also in all tissues and organs of adult organisms. Adult stem cells (ASC) are considered to be a peculiar regeneration reserve of organisms and became the hope of regenerative medicine. Using of adult stem cells gives the possibility of carrying out autologous transplantations, where donor and host is the same individual. Autologous grafts should not induce adaptive immune response, which is the most serious barrier of transplantology. Problems which limit routine use of adult stem cells in regenerative medicine reslult from relatively low number of these cells and objective difficulties with isolation of many ASC types (for example cardiac or nervous stem cells) [1]. However, some populations of adult stem cells, for example those derived from skeletal muscles (SM) or bone marrow (BM) are relatively easy to iso-

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Abbreviations: MDSC – muscle-derived stem cells; MMDSC – multipotential muscle-derived stem cells; MD-HSC – muscle-derived hematopoietic stem cells; MSC – mesenchymal stem cells; PSC – pluripotent stem cells; SC – stem cells; ASC – adult stem cells; BM – bone marrow; SM – skeletal muscle; SMSP – skeletal muscle side population; BMSP – bone marrow side population; EP – early preplate; pp(1-6) – preplate (1-6); ABCG2 – ATP-binding cassette subfamily G member 2.

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late and are considered as promising material for autologous transplantations. Obtaining a muscle biopsy or bone marrow sample from both human or animal is an easy and low invasive procedure. These cells are myogenic, what enables working on new methods of treatment muscle diseases. Surveys regarding the clinical application of myogenic cells refer mainly to muscle dystrophies, myocardial infarction, urinary incontinence or local traumatic injury of muscle.

Myogenic stem cells

Myogenic stem cells are cells with the ability to differentiate into muscle fibers. There are still a lot of controversies concerning origin, potential and characteristic features of different subpopulations of this heterogenous group. The most important populations of cells which are recognized as myogenic stem cells are: satellite cells, multipotential muscle derived stem cells (MMDSCs) and bone marrow mesenchymal stem cells (BM-MSCs) called also multipotent mesenchymal stromal cells (the last name is recommended by International Society for Cellular Therapy [3]. There is also a recently described population of pluripotent stem cells (PSC) residing in adult tissues, which also have myogenic potential and can contribute in muscle regeneration [4].

Satellite cells

Satellite cells are undifferentiated, mononuclear cells located between basal lamina and sarcolemma of muscle fiber. For the first time they were described by Alexander Mauro in 1961. At the beginning of 90-ties this population seemed to be well recognized, but within the last decade a lot of controversies around satellite cells have appeared. Currently it is clear, that the location between basal lamina and sarcolemma does not unambiguously characterize the cell type. It is heterogenous group either concerning phenotype, origin or the level of differentiation. However, one main population can be marked off within this group. This population will be here called "typical" satellite cells. During embryonic development "typical" satellite cells migrate from the dorsal compartment of the somite – dermomyotome [7]. The transcription factor Pax7 is considered to be the key protein conditioning the presence and myogenic determination of "typical" satellite cells. Seale *et. al.* [8] described the complete absence of satellite cells in animals without expression of Pax7. However, the subsequent publication of Oustanin et al [9] revealed, that muscle of *pax7-/-* mice contain a reduced but substantial number of satellite cells. At the same time, muscle regeneration was impaired in adult *pax7-/-* mutant mice what confirmed the essential role of Pax7 in myogenesis. Moreover, it is thought that the presence of Pax7 is required for MyoD expression [10], but in animals *pax7-/-* this function can be, at least partially, taken over by Pax3 protein [11]. Besides Pax7, "typical" satellite cells express also M-cadherin, c-met, foxk1, CD34 [12-14]. Satellite cells possessing such a phenotype are generally considered as unipotent stem cells with the ability to differentiate into myogenic, but not any other cell linage. These cells constitute the regeneration pool of postmitotic muscle fibers. In normal conditions satellite cells remain quiescent, but muscle injury activate them. They start proliferating and expressing muscle regulatory factors (MRF) proteins – Myf5 and MyoD. The proliferative phase takes about 2-5 days following injury. In this time myoblasts (satellite cells progeny), expand in response to stimuli released by damaged muscle fibers and inflammatory cells recruited to the area. The process is also modulated by extracellular matrix proteins. Eventually, about 5-7 days following injury, myoblasts withdraw from cell cycle and begin to differentiate which is conditioned by expression of myogenin and finally – myosin heavy chain (MyHC) [15]. They can fuse with each other creating new fibers or joining already existing ones. The proliferative potential of satellite cells is quite remarkable. It was demonstrated that as few as seven satellite cells associated with one myofiber can regenerate a hundred or more new myofibers containing thousands of myonuclei [16]. Apart from ability to proliferate and differentiate satellite cells can divide asymmetrically to reestablish a pool of residual undifferentiated cells within the muscle [16,17]. This capacity is very important because it decides about numbering satellite cells among adult stem cells. Satellite cells are one of few populations of stem cells which can be relatively easy isolated from an adult organism – obtaining a piece of skeletal muscle weighting few grams is an invasive, but not very upsetting intervention. Moreover, satellite cells are comparatively numerous group – approximately 2-5% of nuclei in muscle of adult mouse belong to this population [18]. These features decide about considering satellite cells as a good material for autologous transplantations in cases of muscle dysfunction.

Multipotential muscle-derived stem cells (MMDSC)

During last decade of the 20th century it was found out that a population of undifferentiated cells in skeletal muscles is more heterogenous then it was previously thought. The presence of cells with the potential of mesenchymal stem cells was revealed in muscles of birds, rabbits and human beings [19]. Depending on isolation method or described features they are called either muscle-derived stem cells (MDSCs) [20], skele-

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Table 1. Characterization of multipotential muscle-derived stem cells obtained by different methods of isolation.

Type of cells	Method of isolation	Cell surface markers	Differentiation potential in vitro	Differentiation potential in vivo	Author
Muscle SP cells	Adhesion properties (pp2-pp3) followed by Hoechst 33342 staining	Sca-1 + c-kit + CD45 -	not determined	H: +	Jackson *et al.* 1999 [23]
Muscle SP cells	Staining with Hoechst 33342	Sca-1 + c-kit + CD34 + CD45 -	not determined	not determined	Meeson *et al.* 2004 [24]
Muscle SP cells	Staining with Hoechst 33342	Sca-1 + CD45 +/-	M: - H: +	M: + H: +	Asakura *et al.* 2002 [21]
Muscle-derived stem cells (MDSCs)	Adhesion properties	Sca-1 + CD34 + Desmin <20%	M: + H: n.b.	M: + H: n.d.	Deasy *et al.* 2005 [25]
MDSCs	Adhesion properties (pp6)	Sca-1 + c-kit – CD45 – CD34 + Desmin + MyoD +/-	M: + O: + H: n.d.	M: + (im, iv) O: + H: n.d.	Lee *et al.* 2000 [25]
MDSCs	Adhesion properties (pp6)	Sca-1 + CD34+ Desmina 10%+	M: + H: +	M: + (iv) H: n.d.	Torrente *et al.* 2001 [28]
MDSCs	Adhesion properties (pp4-pp6)	Sca-1 +/- CD34 +/- Desmin + (80-90%)	M +	M: + (im)	Jankowski *et al.* 2001 [26]
MD-HSC	Spin in percol density gradient (70/40%)	Sca-1 + c-kit + CD45 + CD34 +	not determined	M: + H: +	McKinney-Freeman *et al.* 2002 [22]

M – myogenic potency, H – hematopoietic potency, O – osteogenic potency, im – intramuscular injection, iv – intravenous injection, n.d. – not determined

tal muscle side population (SMSP) [21], or muscle-derived hematopoietic stem cells (MD-HSCs) [22]. It is not clear whether these cells are differently called but are in fact the same population or if they are rather distinct groups of cells. In recent years several trials were performed in order to fully characterize undifferentiated cells situated in skeletal muscles. In any case, many points concerning their origin, potency and functions remain to be elucidated.

To describe muscle stem cell phenotype the presence of stem cell antigen-1 (sca-1), CD34 (marker of hematopoietic and satellite cells), CD45, c-kit (markers of hematopoietic cells) and desmin (marker of myogenic cells) were determined (Table 1). Conflicting data were reported [21-28], what can suggest that several distinct populations of multipotential muscle-derived stem cells exist. On the other hand, obtaining various expression profiles could be caused by using different methods of protein detection – western blot, immunocytochemistry or flow cytometry. Finally, depending on the cell status (quiescent, proliferating, differentiating, apoptotic) the protein expression change dynamically in cells derived from muscle explants what can be another explanation of diversity of results [29-31]. The results were consistent merely with regard to sca-1 expression – all studies demonstrated that multipotential muscle derived stem cells are positive for this protein.

Generally, two different methods of MMDSC isolation can be distinguished. The first method, described by group of researchers from Pittsburgh, is based primarily on differences in adhesion properties of cells [32]. Cells obtained by enzymatic digestion of muscle tissue are seeded on a culture dish and after 1 h the medium and nonadherent cells are transferred to another dish (preplating). Next, analogous preplates are repeated at 24 h intervals until preplate 6 (pp6) is completed (Fig. 1B). The cells which rapidly attach to the surface are mainly fibroblasts (pp1), cells which adhere within 24-48 h are predominantly satellite cells

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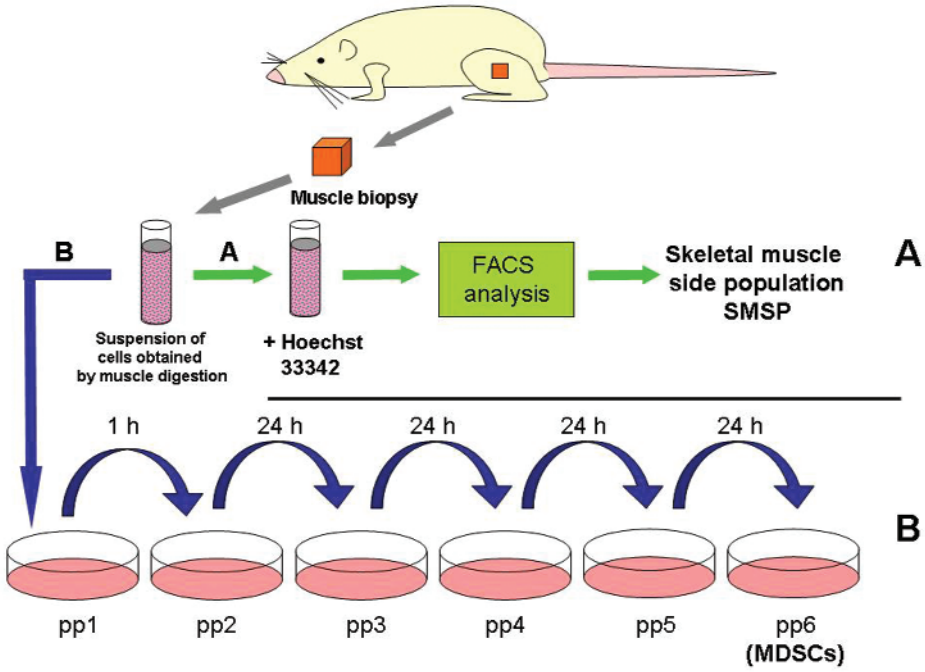

Fig. 1. Two methods of MMDSC isolation. The muscle explants are minced and digested. **A**. Cells released from the muscle tissue are stained with Hoechst 33342 and analyzed by FACS. Cells which display increased dye efflux are called skeletal muscle side population (SMSP). **B**. The technique based on adherence properties – cells are seeded at a flask and after 1 h the medium and non-adherent cells are moved to another dish. Analogous preplates are repeated at 24 h intervals until preplate 6 (pp6) is completed. This population is called MDSC.

(pp2 – pp4) and the population which settle the most slowly on a flask consist multipotential stem cells (pp-6) [32]. The phenotype of fraction pp6 was described as sca-1+, CD34+, CD45-, c-kit- with the expression of desmin on a different level. This population is called muscle-derived stem cells (MDSC). MDSCs cultured *in vitro* differentiate spontanously into myotubes, but also, when appropriately stimulated (by addition of growth factors to the medium or transduction with certain genes) these cells can give rise either to osteoblasts [27], chondroblasts [33], hematopoietic cells [18] or endothelial cells [34]. Additionally, it was shown, that population of sca-1+, CD45-, c-kit- SM-derived cells display also cardiomyogenic potential [6] however, the higher cadriomyogenic potency was demonstrated in sca-1- subpopuation of SM-derived cells. Muscle-derived stem cells participate into muscle regeneration following intramuscular injection. Interestingly, they were also found in muscles after intravenous administration, and their number was higher in muscles previously injured then in control ones [28]. Alternatively, MDSCs can contribute into bone or cartilage repair, but only after transduction with BMP-2 or BMP-4 (bone morphogenetic protein) [27,33].

To sum up, cells called MDSC can be recognized as multipotential, but with distinct tendency to myogenic differentiation. Presented results indicate that the poorer cells adhere to the flask surface the more undifferentiated state they have. The expression of desmin gradually decrease with subsequent preplates and in pp6 population only about 10-20% of cells are positive for this protein [25,28]. However, pp6 cells cultured in standard conditions spontaneously enter the myogenic pathway what is associated with increased desmin expression [32]. It explains results obtained by Lee *et. al.* [27] and Jankowski *et. al.* [35] who have demonstrated that the majority of MDSCs was positive for desmin. Studies regarding cell viability after transplantation have shown that more MDSC survive following intramuscular administration comparing to more differentiated cells (myoblasts). After injection of the same number of either MDSC or early preplate (EP) cells, the contribution to muscle regeneration 30 and 90 d later was even 10 time higher in MDSC group. Marked differences were probably associated with the distinct immunogenicity between MDSCs and EP cells. The evaluation of MHC-1 expression on cell membranes of both cell types revealed that MHC-1 was present on 63% of EP cells whereas only on 0.5% of MDSCs. However, MDSC is a much less numerous cell population comparing to "typical" satellite cells which dominate in EP group. Only one clone of MDSC can be obtain from 10^5 of cells originally isolated from muscle tissue [32].

The second, frequently used method of isolation of muscle-derived multipotential stem cells is sorting them by flow cytometry after staining with Hoechst 33324 (Fig. 1A). Cells obtained with this technique are called skeletal muscle side population (SMSP). Unfortunately, the description "side population" characterize only the ability to efflux the dye and does not define the features or function of these cells. It is considered that ATP-binding cassete (ABCG2) protein is respon-

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sible for the phenotype of SP cells [36,37]. Initially, the term "side population" was associated mainly with a subpopulation of hematopoietic stem cells [38]. However, later studies revealed that high level of ABCG2 expression and effluxing the Hoechst 33342 dye characterize some types of stem cells in other tissues and organs (for example skeletal muscle tissue, mammary gland or lungs [21,39,40] and also some cancer cells [41]). Therefore, side population phenotype is rather associated with low level of differentiation then certain features or origin of cells.

Skeletal muscle side population has been characterized as sca-1+, CD34+, CD45+/-, c-kit+/-, desmin- [18,21]. SMSP cells are spherical and poorly adhere on the dish surface [42], in which they resemble MDSC population. Evaluating of differentiation potential revealed that SMSP can give rise into all hematopoetic lines both *in vitro* and *in vivo* [21,23]. The question of myogenic potential is more complex. Asakura *et. al.* [21] demonstrated that SMSP cells harvested *in vitro* do not differentiate spontaneously into myocytes. However, the co-culture of lac-Z-expressing SP cells with primary myoblasts caused the formation of small number (3.8%) of multinucleated myotubes that coexpressed lacZ and desmin. These results indicate that cell-mediated inductive interactions are needed to induce myogenic potential of SMSP. Interestingly, only CD45- subpopulation of SP cells possesses such a capability – CD45+ SP cells do not undergo myogenic specification even when co-cultured with myoblasts. Myogenic potential of SP was also tested in the *in vivo* studies. Side population cells contribute to new fibers formation after injecting into muscle injured with cardiotoxin. Moreover, they can be found in the position of satellite cells after transplantation. However, CD45- subpopulation cells differentiate into muscle fibers in a higher percent then CD45+ cells, what confirm *in vitro* studies [21,22]. Regardless of hematopoietic and myogenic potential, SMSP display also endothelial trait (CD31+) [43]. Moreover, it was shown that SMSP expresses angiopoietin 2 and the Tie2 receptor, which is bound and activated by angiopoietins [24]. It means, that most of the SMSP cells share partially signaling pathways with endothelial/hematopoietic precursor cell populations.

In terms of myogenic specification of SMSP cells, interesting observation was done by Asakura *et. al.* [21]. They demonstrated that lacking of *Pax7* gene in experimental animals does not influence the number of muscle SP cells. Furthermore, *Pax7-/-* SP cells can differentiate into myotubes when cocultured with myoblasts what indicate that *Pax7* gene is not required for myogenic specification of SMSP cells. Finally, forced expression of MyoD induced myogenic differentatiation of *Pax7-/-* SP cells but not *pax7-/-* myoblasts [21]. All this data suggest that SMSP and satellite cells are distinct populations and probably have different origin. On the other hand, results of Seale *et. al.* [10] indicate, that Pax7 is necessary and sufficient for the myogenic specification of CD45+, sca-1+ muscle-derived cells (subpopulation of SMSP). Therefore, to elucidate the role of Pax7 in myogenesis of SMSP cells, further studies are required.

Pluripotent stem cells (PSC) from adult tissues

One more type of cells is postulated as potentially contributing to regeneration of skeletal muscles. This is a rare population called very small embryonic like (VSEL) stem cells. These cells were identified by group of researchers from Louisville [4]. It was found that VSELs express markers of pluripotent stem cells such as SSEA-1, Oct-4, Nanog and Rex-1 and Rif-1 telomerase protein. As it was mentioned before, pluripotency means the ability to differentiate into cells from all three germ layers. Therefore, VSEL stem cells display also myogenic potential. This is hypothesize that PSC are deposited during gastrulation and organogenesis in developing organs/tissues. They were found for example in adult bone marrow, lungs, myocardium, liver [2]. Until now, it was not demonstrated that VSELs are present in adult skeletal muscles, however, another group of researchers found in a murine SM a rare population of cells which displayed myogenic, adipogenic and neurogenic potential, what suggest pluripotency [5]. Moreover, the expression of several markers of PSC (Oct-4, Nanog, Rex-1, Rif-1) were identified at transcript level in cells isolated from skeletal muscles [6]. The expression of PSC markers was higher in sca-1- subpopulation of SM-derived cells then in sca-1$^+$ cells.

Origin of myogenic stem cells

It has been established that "typical" satellite cells (committed muscle progenitors) originate from the dorsal compartment of the somite, the dermomyotome and that Pax3/Pax7 are the key factors to direct embryonic cells into myogenicity [7,44]. The issue of origin of multipotential muscle-derived stem cells is more problematic. Schienda *et. al.* [45] demonstrated that at least part of SMSP cells arise from hypaxial somite and, similarly to satellite cells, they are localized in skeletal muscle as early as during embryonic period. On the other hand, numerous studies indicate that SMSP are derived from bone marrow. It has been shown, that both acute and chronic injury of muscle result in mobilization of mesenchymal stem cells from bone morrow to the peripheral blood [47]. Circulating bone marrow-derived cells are able to settle in skeletal

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Table 2. Comparison of "typical" and other muscle satellite cells.

	„Typical" muscle satellite cells	Other types of muscle satellite cells
Origin	Dorsal compartment of the somite - dermomyotome	Different possible origins: a. somitic, b. deposited in tissue during early gastrulation (derived from epiblast stem cells), c. supplemented throughout life from bone marrow cells by circulation (originating from MSC, HSC or VSELs)
Differentiation potency	Unipotential – give rise only to myogenic cells	Multipotential or unipotential
Phenotype	$Pax7^{+}$, M-cadherin^{+}, c-met^{+}, CXCR4^{+}, foxk1^{-}, CD34^{-}, desmin^{-}	$Pax7^{+/-}$, M-cadherin$^{+/-}$, desmin$^{+/-}$, CD34$^{+/-}$

muscle and contribute to their regeneration [18,42,48-50]. Intravenously injected bone marrow cells were found in muscle even 13 years after transplantation [51]. From the other side, intravenous administration of male-derived unfractionated bone marrow cells into female mdx mice resulted in formation of dystrophin-expressing Y-chromosome-positive muscle fibers already after 8 weeks [42]. Following analogous injection of the same number of bone marrow main population (BMMP) and bone marrow side population (BMSP) the regeneration of muscle was 100 fold more effective in the case of BMSP transplantation [52]. This suggests that stem cells finding niche in skeletal muscle during postnatal life are derived mainly from bone marrow side population. However, as mentioned above, the term "side population" does not specify the type of cells in details. Considering the fact, that some of multipotential muscle-derived stem cells (MMDSCs) display expression of hematopoietic marker CD45, and the other part is negative to this protein, it is likely, that both hematopoietic and mesenchymal bone marrow stem cells can migrate into skeletal muscle and constitute, at least in part, MMDSCs population.

The mechanism, which determines mobilization of cells from bone marrow and homing them in the muscle tissue is not completely elucidated. Generally, it is recognized, that damaged muscle release signals (chemokines, growth factors), which, on the basis of chemical gradient, attract circulating stem cells (SC) to the injured area [53,54]. Up to date, the role of several factors has been demonstrated in homing of circulating SC to the muscle tissue. Among them are hepatocyte growth factor (HGF), stromal-derived factor-1 (SDF-1), leukemia inhibitory factor (LIF), insulin-like growth factor-1 (IGF-1) [53-55]. The presence of appropriate receptors (c-met, CXCR4, LIF-R) on a cells surface allows the release from the bone marrow and decide about trafficking certain cell type to the area with the high concentration of corresponding ligand. Regarding to the muscle tissue, CXCR4/SDF-1 axis is suggested to play a crucial role in settlement of non-hematopoietic stem cells in skeletal muscles [53,56], whereas hematopoietic stem cells are probably trafficked by the c-met/HGF axis [54]. The presence of CXCR4 receptor on hematopoietic stem cells and the fact, that muscle tissue is a SDF-1 positive stem cell niche suggest, that CXCR4/SDF-1 interaction can also contribute to homing of HSC in muscle [57], however, the results of Rosu-Myles *et. al.* [54] does not confirm this hypothesis.

The migration of BM-derived SC outside from the blood vessel is possible due to the presence of adhesion molecules as integrins or selectins on the cell surface [58]. There are some factors influencing stem cells homing. It has been demonstrated, that regenerating muscle incorporate more circulating cells then noninjured one [18]. Interestingly, also physiologic stresses encountered by healthy organisms throughout life enhance homing of circulating SCs in the muscle [59]. Moreover, it was demonstrated, that the level of incorporation of BM cells depends also on muscle type [60].

Bone marrow derived cells, which settled in the muscle tissue, gradually change their phenotype and differentiation potential under the impact of local signals released by myogenic environment. McKinney-Freeman *et. al.* [49] analyzed the phenotype of CD45+ cells isolated directly from bone marrow in comparison to the same cell population after homing in the muscle. They reported considerable downregulation of c-kit protein in the settled cells (over 1000-fold) and significantly lower potency to repopulate hematopoietic lineages (22-fold). In another study, comparative microarray analysis of BMSP and SMSP revealed that expression of over 50% of examined genes differed in these two populations [15]. Moreover, long term studies demonstrated, that BM-derived cells can be localized with time in the position of satellite cells and are positive for M-cadherin [50] (The comparison of "typical" and other types of muscle satellite cells is summarized in Table 2).

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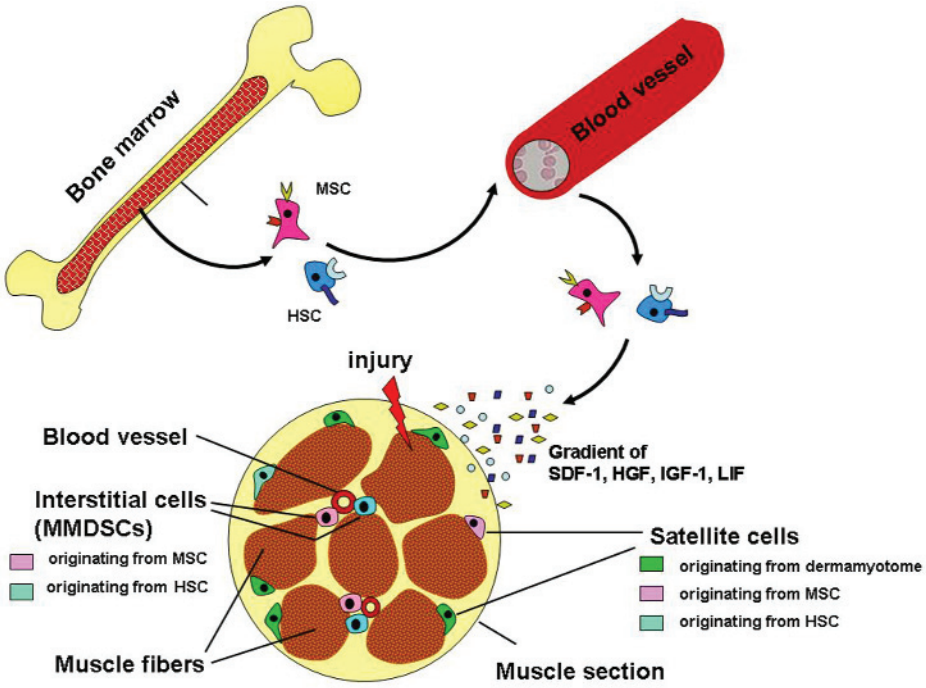

Fig 2. Proposed relationship between BM-derived stem cells, MMDSC and satellite cells. Both HSC and MSC are released from bone marrow to the peripheral blood as an answer to certain stimuli (for example exercises or injuries). They are trafficked by gradient of chemokines or growth factors and settle in various tissue and organs (in this case in skeletal muscle). At the beginning they are located in interstitial tissue in the neighborhood of blood vessels and at this level can be classified as multipotent muscle stem cells. Their phenotype is gradually changed and determination into myogenic pathway increases. With time, MMDSCs can enter satellite cell position and finally are able to become muscle precursor cell expressing Pax7. Nevertheless, the large majority of satellite cells (more then 95%) are cells originating from dermamyotome.

There is an agreement, that "typical" satellite cells originating from dermamyotome constitute the basic regenerative reserve of skeletal muscle tissue which is present already in the prenatal period. However, all data cited above indicate, that this "somitic" pool is supplemented throughout life by cells derived from bone marrow (both hematopoietic and mesenchymal). These cells are mobilized and trafficked during exercises, injuries and probably other conditions which remain unknown until now. Initially, BM-derived cells settle in the interstitial tissue in the neighborhood of blood vessels and display relatively low myogenic potential. Yet, the environment of regenerating muscle enhance myogenic determination, so, with time, cells derived primarily from bone marrow or their progeny can enter satellite cells position and begin to express markers of myogenic precursors [10,18,50,61]. The settlement of BM-derived cells in skeletal muscles has been schematically illustrated in Fig. 2. It is likely, that MDSCs obtained by using preplating method are the progeny of BM-derived cells on succeeding levels of myogenic differentiation. Therefore, subsequent subpopulations (from pp6 to pp2) display the rising expression of myogenic marker – desmin, and the lowering expression of undifferentiated cells marker – sca-1. [25,28]. Interestingly, not all cells located in satellite cell position are terminally determined to myogenic differentiation [62]. Therefore, we propose the following sequence of events: first, MDSCs reach the localization beneath basal lamina and afterwards they can acquire (not necessarily) the phenotype of myogenic precursors. Such a hypothesis can explain results, where mesenchymal potential of satellite cells was described [31,62], although they are generally considered to be determined myogenic precursors. In the study of Sherwood *et. al.* [18] bone marrow cells were transplanted retroorbitally, and the effect was evaluated after 4 – 12 weeks. Grafted cells were found in skeletal muscles both in interstitial tissue and in the position of satellite cells. Additionally, among cells isolated from muscle explants, the population with the highest myogenic activity was analyzed. These cells displayed $CD45^-$, $sca\text{-}1^-$, $Mac\text{-}1^-$, $CD34^+$, $CXCR4^+$, $\beta1\text{-integrin}^+$ characteristics. Interestingly, cells with such a phenotype were not found among the population derived from BM and settled in the muscle. Thus, BM-derived cells can not (at least within 12 weeks) reach the phenotype of typical muscle progenitors. On the other hand, LaBarge *et. al.* [61] demonstrated, that the contribution of bone marrow-derived satellite cells to muscle regeneration increased in animals after 6 months of exposure to a running wheel. The number of new muscle fibers created with participation of BM-derived cells arise up to 3.5% of all myofibers (comparing 0.16% in non-exercised control animals).

Importantly, BM-derived cells homed in muscle tissue contribute not only to the regeneration of muscle fibers. It was demonstrated, that BM-derived $CD45^+$, $sca\text{-}1^+$ cells isolated from skeletal muscle and transplanted to another muscle took part in a vascular regeneration (both of endothelium and smooth muscle layer) [48].

It was demonstrated, that beside hematopoietic and mesenchymal stem cells, also small amount of pluripo-

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tent stem cells can be mobilized from bone marrow into peripheral blood during skeletal muscle damage [46] VSEL stem cells are considered to be a mobile pool of primitive stem cells which can contribute to regeneration of different tissue and organs depending on the current needs.

Muscle regeneration potential during aging

Myogenic stem cells become a new material for autologous transplantations in human patients. Therefore, the question about features and potential of this population in aging organisms seems to be very important. It is known, that the muscle regeneration capability decrease with age [63]. The mechanism of this impairment has not been completely elucidated yet. In term of clinical applications it is crucial to establish what is the predominant cause of declining regenerative potency of skeletal muscle. Are these an age-related changes in number or features of myogenic stem cells (cell intrinsic factors) or rather alterations in the surrounding environment (cell extrinsic factors)?

The results of studies concerning the correlation of age with the number of muscle stem cells (usually satellite cells) are unequivocal. Depending on the examined species, type of muscle or methodology which has been used, the number of muscle stem cells was decreased, maintained or it increased with age [64]. There are studies indicating that, not the quantity, but the appropriate activation and efficient proliferation before terminal differentiation has the key impact on regeneration ability of skeletal muscle. It has been suggested that changes in the expression of Noth and Wnt factors can be important in aging muscle. It was shown that the regulation of Notch signaling controls satellite cell activation and proliferation in postnatal myogenesis [65], whereas the initiation of differentiation is Wnt dependent. In aged muscle, Wnt signaling is increased in early activated satellite cells [66] and, at the same time, the activation of Notch receptor is impaired [67]. This results in premature shifting from proliferation phase to differentiation and finally leads to less regeneration ability in aging organisms. However, it was observed, that this trend is reversible, thus, depends on extrinsic factors. Exposure of aged satellite cells to young systemic environment restored appropriate activation of Notch receptor and prolonged proliferation phase [68]. Another significant age-related feature of satellite cells is increased tendency to convert to fibroblasts in the *in vitro* culture. Yet, also in this case, exposure of aged cells to serum derived from young animals caused the decline of myogenic-to-fibrogenic conversion. Thus, increased fibrosis observed in aging muscle seems to be related to the environmental conditions. Others extrinsic changes which are considered to influence the regeneration capacity are reduced skeletal muscle capillarization [69] and thickening of basal lamina surrounding the muscle fibres [70] in aged compared to young organisms. Both of these factors can hinder the access of different stimuli to the muscle and can have an impact on settlement and fate of mesenchymal stem cells derived from BM [71]. To summarize, most of studies indicate, that extrinsic influences play predominant role in age-related impairment of muscle regeneration. The most distinct confirmation of such a hypothesis are experiments with using heterochronic parabiotic pairs of animals (where the circulation of young and old animal is crossed by surgical joining). It was demonstrated that the muscles of older partner in such a pair displayed enhanced regeneration capability and decreased fibrosis. The effect observed in young animal was opposite [66]. Nevertheless, the cell intrinsic changes in aging muscle are not meaningless. Among irreversible cell features influencing impaired regeneration potential of aging muscle the most important seems to be telomere shortening and increased tendency to undergo apoptosis [64].

Mesenchymal stem cells

As mentioned before, bone marrow mesenchymal stem cells (BM-MSCs) are also considered as myogenic stem cells. Mesenchymal stem cells have been recognized to be multipotent, what means the ability to differentiate into all cell types originating from one germ leyer (mesodermal in this case). Indeed, it was demonstrated that MSCs can give rise to osteoblasts, chondroblasts, adipocytes [72] and also skeletal muscle cells [73,74], cardiomyocytes [75,76] and smooth muscle cells [78]. The ease of obtaining bone marrow sample and myogenic potential of MSCs make this population an attractive candidate for cellular transplantation in cases of diseases associated with muscle dysfunction [77]. However, there are still a lot of controversies around the level of myogenic potential of mesenchymal cells. Some researchers claim that the whole population of MSCs can be differentiated into myoblasts, if appropriately treated [73], whereas other authors indicate, that only little proportion of BM-MSCs displays myogenic potential [79-81]. Numerous studies were focused on the methodology of induction of MSCs differentiation into muscle cells. First reports pointed to the DNA methyltransferase inhibitor – 5-azacitidine as the factor initializing myogenesis of MSCs [82]. Some studies confirm, that the addition of 5-azacitidine to the culture medium induces the expression of myogenic cells marker – desmin [75] or cardiomyocytes markers – β-myosin heavy chain and cardiac troponin-T [76,83] in harvested MSCs. On the other hand, there are reports questioning such an effect of 5-azacitidine. Chan *et. al.* [84] tested different con-

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centrations of this factor and did not observed expression of any of myogenic markers but massive death of treated cells. Similarly, Liu *et. al.* [85] demonstrated no expression of cardiomyocytes markers in MSCs exposed to 5-azacitidine. Finally, studies of Balana *et. al.* [86] indicated that 5-azacitidine induces differentiation of MSCs into neither myoblasts nor cardiomyocytes, but can change electrophysiological properties of treated cells. Another factor proposed as potential inducer of myogenic differentiation of MSCs is galectin-1 [84]. Previously, it was shown that galectin-1 is capable to convert mouse dermal fibroblasts to the myogenic lineage [87]. Up to date, an effect of galectin-1 activity on mesenchymal stem cells was not confirmed by other researchers. Regarding myogenic potential of BM-MSCs, an interesting data were reported by Dezawa *et. al.* [73]. They described the methodology, which allows for differentiation of primary pool of MSCs into muscle cells with efficacy of 89%. To obtain such a high differentiation rate, MSCs were cultured in a medium containing mixture of cytokines and growth factors (bFGF, forskolin, PDGF i neuregulin) and subsequently transfected with gene encoding Notch 1 intracellular domain (NICD). Following this procedure, cells described as muscle-MSCs (M-MSCs) expressed myogenic markers (Myo-D, myogenin) and fused into myotubes (fusion index amounted to 20%). Moreover, M-MSCs were positive for transcription factor Pax7 and c-met receptor, which are well known specific markers of satellite cells.

Alternative approach to induce myogenesis in MSCs is the exposure of these cells to myogenic environment. Studies verifying this conception were performed both *in vitro* [74,88] and *in vivo* [89]. It is known, that cells release cytokines to the surrounding environment and can modify the fate of neighboring cells in a paracrine way. Additionally, changes in genes expression can be induced by direct cell-to-cell interactions. Indeed, GFP (green fluorescent protein) positive mesenchymal stem cells co-cultured with cardiomycytes or satellite cells differentiated in either cardiac cells [88] or myotubes [74] respectively. These results confirm both the significance of cell-to-cell interaction and the presence of myogenic potential of MSCs. However, the differentiation rate in these conditions was highly limited. Among multinucleated myotubes only 1-2% revealed the expression of GFP. The fate of mesenchymal stem cells injected into either skeletal or cardiac muscle was also analyzed. It was demonstrated that undifferentiated MSCs can undergo myogenesis after intramuscular administration, but similarly to the *in vitro* studies, the proportion of differentiated cells was barely detectable – only 0,44% of transplanted MSCs fused in myotubes [90]. Interesting study was conducted by Nassiri *et. al.* [89] where the efficacy of MSCs transplantation with and without prior differentiation was compared. Cells were injected around the myocardial infarction are experimentally induced in a rabbit model. Improvement in left ventricular function, vascular density and reduction of infarcted area did not differ significantly between two groups. The perspective to transplant undifferentiated mesenchymal stem cells seems to be promising, because it does not require time-consuming and expensive extracorporeal manipulations on cells. On the other hand, the more undifferentiated the cells are, the higher risk of neoplastic transformation exists [91]. Nonetheless, further studies are needed to confirm the results obtained by Nassiri *et. al.* [89].

Conclusions and perspectives

Clearly, the biology of myogenic stem cells is very complex. There are still a lot of questions which remain to be elucidated. It is important to establish in what extent BM-derived muscle stem cells support physiological regeneration of skeletal muscle. Are they significant players or rather have marginal meaning? Moreover, how can we influence the level of mobilization of bone marrow cells and their settlement in the muscle tissue? Multipotential MDSC seems to be a promising population, but its rarity seriously limits the possibility of clinical use. The same, but in even wider extend, refers to pluripotential stem cells. Therefore, further work on improving the techniques of isolation and expansion of MMDSCs and adult PSC should be conducted. In term of future clinical applications the choice of the best cell type for transplantation should be established. There is a variety of conditions where the transfer of myogenic stem cells can be good for medical practice (for example: muscular dystrophies, myocardial infarction, urinary incontinence). Therefore, it is likely, that for each of dysfunction, different cell type can be the best option. Due to real perspective of performing autologous myogenic stem cells transplantations in human patients it is crucial to understand more profoundly age-related changes in regeneration capacity of muscle. Such a knowledge allows for elaboration novel strategies of improving cellular transfer in older patients.

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