Special Issue: Mesenchymal stem cells

Review Article

Pluripotent stem cell as a source of mesenchymal stem cell

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Mesenchymal stem cell (MSC) is one of somatic stem cells useful for regenerative medicine. This type of stem cell can be easily isolated form adult tissues such as bone marrow (BM). However, MSC isolation is difficult in elder patients and self-MSC cannot be prepared quickly. ES cells, a pluripotent stem cells (PSC), have recently been characterized as a novel source of MSCs. Furthermore, medical progress found a new method of self-PSC generation in which somatic cells can be reprogrammed into pluripotent stem cells, namely induced pluripotent stem cells (iPSCs). In this review, I provide and discuss our recent results regarding to ES/iPS cell-derived Mesodermal cells and MSCs. Based on this information; I will describe future perspectives for the utility of MSCs and ES/iPS cell-derived MSCs.

Rec.11/12/2012, Acc.12/17/2012, pp19-28

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Key words embryonic stem cells, induced pluripotent stem cells, mesoderm

Introduction

Stem cells are implicated in developing, maintaining and repairing the tissues. They have a potential to differentiate into multiple cell types and undergo sustained growth with their self-renew. In addition, stem cells have been applied for clinical medicines such as bone marrow transplantation (BMT). The transplantation of hematopietic stem cells (HSCs), called as BMT, greatly improve the prognosis of malignant blood diseases. The therapeutic strategy of HSCs is based on the replacement of bone marrow by normal stem cells after removing abnormal malignant cells. As BMT cures the patients with malignant blood diseases, stem cell therapy is generally expected to be effective on the intractable diseases.

Mesenchymal stem cell (MSC) is one of somatic stem cells useful for clinical therapy[1]. This type of stem cell can be isolated form adult tissues such as bone marrow (BM)[2]. Furthermore, induced pluripotent stem (iPS) cells have recently been characterized as a novel source of MSCs. In this review, I provide and discuss our recent results regarding to ES cell-derived Mesodermal cells and MSCs. Based on this information, we will describe future perspectives for the utility of MSCs and ES/iPS cell-derived MSCs.

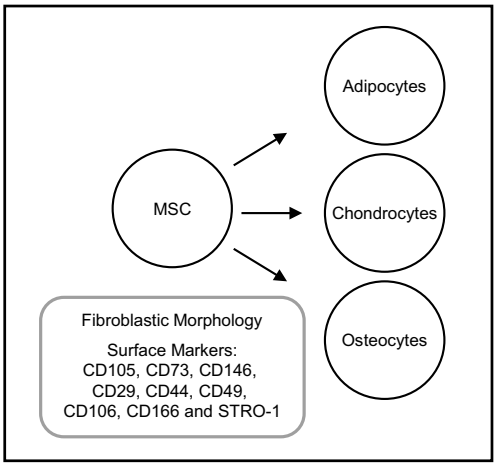

Fig.1 Definition of mesenchymal stem cell

Mesenchymal stem cell (MSC) exhibits fibroblastic morphology and expresses CD105, CD73, CD146, CD29, CD44, CD49, CD106, CD166 and STRO-1. MSC can give rise to three principle lineages including adipocytes, chondrocytes and osteocytes.

Mesenchymal stem cell

In the 1970s, MSC was initially discovered as a rare population of adherent cells in BM[3]. The adherent cells were characterized by a capacity to form individual colonies from single cells, which were termed colony-forming unit fibroblasts (CFU-Fs). These cells proliferated and have a potential to differentiate into mesenchymal lineages such as osteoblasts[3, 4]. Subsequently, Caplan et al. categorized the cells as "mesenchymal stem cells (MSCs)" due to their ability to differentiate into mesenchymal lineages[5]. MSCs are defined by three features; i) fibroblastic morphology, ii) sustained proliferation *in vitro* and iii) differentiation potential into three principle cell types, osteocytes, chondrocytes and adipocytes (Fig.1)[3, 6]. Several study reported that MSCs can differentiate into muscles and tendon, and even into neurons that do not belong to mesenchymal lineages[7]. Although MSCs were originally isolated from bone marrow, recent studies revealed that they widely distributed in various tissues including fat, skeletal muscle, synovium, dental pulp, heart and spleen[1, 8-12]. As MSCs is easily isolated from BM and adipose tissues and have multipotency, a proliferative capacity and low-risk for tumorigenicity, MSCs are anticipated to be a promising cell source for cell-based therapies such as transplantation.

MSCs significantly contribute toward tissue recovery and immune-modulation[13]. They are shown to replace chondrocytes and osteocytes in models of arthritis and bone fractures, respectively[14]. Recent studies demonstrated that MSCs have an ability to modulate immunological response. The immunosuppression is mediated by the injection of MSCs. The treatment with MSCs is effective on Graft versus host disease (GVHD) after BMTs[15]. MSCs can suppress T cells reaction to foreign antigens by the secretary molecules such as interferons and interleukins[13]. This indicates that therapeutic application relies on not only directed differentiation into mesenchymal tissues but also suppressive effect on immune-reaction in diseases.

Mesoderm development in mouse embryos

The development processes in embryogenesis, which have been found by the studies of developmental biology, is useful for drawing out the methods concerning *in vitro* ES/iPS cell culture. Mesoderm, one of three primary germ layers, mainly gives rise to mesenchymal tissues. The inner cell mass at E3.5 in mouse and at E5.0 ～ E7.0 in human gives rise to the primitive endoderm and epiblast, which is the source of the three primary germ layers during gastrulation[16]. The formation of the germ layers, and subsequently their fates, are determined through a process dependent upon spatial and temporal regulatory control. In mouse development, mesoderm starts to be generated at E6.5 and, for a short time, dramatically produces three major types of mesoderm; organizer, embryonic mesoderm and extra-embryonic mesoderm[17, 18]. The most initial mesoderm appears at a proximal region in epiblasts of embryo as an early gastrula organizer (EGO)[17]. EGO migrates into anterior part of embryo and become to mid gastrula organizer (MGO) that contributes to axial mesoderm. While organizer migrates, the epiblast at posterior region subsequently begin to transform to second type of mesoderm; embryonic mesoderm, in primitive streak[19]. Along with the elongation of primitive streak distally, embryonic mesoderm become to diversify region-specifically two types of mesoderms, paraxial and lateral mesoderm, which eventually forms a majority of mesoderm progenies.

Each mesodermal precursor population gives rise to; the prechordal plate and notochord (from axial mesoderm); somites, which develop into muscles, bones and cartilage (from paraxial mesoderm); heart, blood vessels and blood cells (from lateral mesoderm)[16, 18]. The epiblasts at the proximal part of embryo also produce a third type of mesoderm; extra-embryonic mesoderm. The precursors of this

mesoderm move into the nascent streak and migrate to extra-embryonic part in which they mainly give rise to primitive hematopoietic cells and endothelial cells of the yolk sac vasculature[20, 21].

Pluripotent stem cells and their differentiation

Embryonic stem (ES) cells originate from the inner cell mass (ICM) that give rise to the embryo body prospectively, and can be maintained with unlimited growth *in vitro*[22]. ES cells have the potential to broadly differentiate into many cell types, including mesodermal cells and their descendants. As various cells are shown to be induced from undifferentiated ES cells under appropriate culture conditions *in vitro, in vitro* ES cell culture are available as an experimental tool to study embryonic differentiation[23, 24]. The advantages of *in vitro* ES cell culture are as follows: 1)although the number of cells in the mouse ICM or adult tissue stem cells are too limited to be analyzed directly, ES cells are capable of proliferating and providing enough cells. 2)ES cells can also be manipulated genetically *in vitro*, and are available to generate chimeric mice, allowing the analysis of the function of a gene of interest *in vivo*. Because of the ability of pluripotency and unlimited growth *in vitro*, human ES cell lines are expected to be utilized for the promised cell source of regenerative therapies[25]. However, as the fertilized eggs are always destroyed to generate ES cells, people must consider the ethical problems about eggs disruption. In addition, immunological rejection must occur when the cells derived from non-self ES cells are transplanted. Fortunately, recent medical progress has perfectly overcome these problems with prospectively ES cell-based cell therapies. The forced expression of four transcriptional factors, Oct3/4, Sox2, Klf4, and c-Myc have reprogrammed mouse and human fibroblasts, and generated pluripotent stem cells similar to ES cells, namely induced pluripotent stem (iPS) cells[26, 27]. While various tissues are the possible source for iPS cells, iPS cells are shown to be generated from leukocytes in peripheral blood, which are ideal source because of low risk and high accessibility[28]. The iPS cells exhibits high differentiation capacity into various cell types such as blood and neural cells and an easy maintenance with unlimited growth. However, there are several disadvantages of using ES/iPS cells. In *in vitro* ES/iPS cell culture, various cell types are simultaneously generated, complicating any subsequent analysis. As shown in transplantation experiments, undifferentiated ES/iPS cells form teratomas in recipient mice. As the differentiation level of ES/iPS cells is not exactly synchronized in the cultures, insufficient purification of transplanted cells causes the contamination of undifferentiated ES/iPS cells, and subsequently leads to teratoma formation. This tumor formation driven by undifferenatiated ES/iPS cells is one of the major obstacles for the application of the differentiated cells in regenerative medicine. To solve these problems, it is necessary to introduce some steps to visualize the intermediate precursor/progenitor cells using cell surface markers and to purify the cells of an interest by FACS[24]. This step is also a critical for the reproducibility of the induction methods. The visualization of the intermediates can raise the efficiency on the reproducibility of the induction from ES/iPS cells to the cells of an interest because of monitoring the production of the progenitors.

As ES cell differentiation culture does not provide usefully positional information for cell type definition, this system definitely requires visible markers to identify and monitor the intermediates that present on the way of differentiation. There are two methods for cell marking to visualize cell lineages; one is the staining with antibodies against surface markers, another is a genetically manipulated ES/iPS cell. The availability of Vascular Endothelial Growth Factor Receptor 2 (also called VEGFR2, FLK1 and KDR) that marks the subtypes of mesoderm cells with a potential to give rise to hematopoietic cells (HPCs) and endothelial cells (ECs) facilitates our understanding on the developmental pathways of these lineages[29-32]. Another important surface marker involving in mesoderm development is Platelet-derived growth factor receptor alpha (PDGFRα) that is mainly expressed in paraxial mesoderm during mouse embryogenesis[33-35]. We have exploited these markers for dissecting the differentiation course of ES cell-derived mesoderm cells. Our previous results obtained from *in vitro* ES cell culture shows that PDGFRα^+VEGFR2$^+$ cell (DP) that initially appears at day 3.5 ES cell culture is a common precursor for PDGRFRα^+VEGFR2$^-$(PSP) and PDGFRα^-VEGFR2$^+$(VSP) cells[36]. Based on the results of *in vitro* fate analysis, we found a new differentiation pathway in which the DP gives rise to both the PSP and the VSP that eventually differentiate into bone and cartilage cells, and HPCs and ECs, respectively (Fig.2)[36]. These indicate that PSP and VSP populations represent the paraxial and lateral mesoderm populations in actual mouse

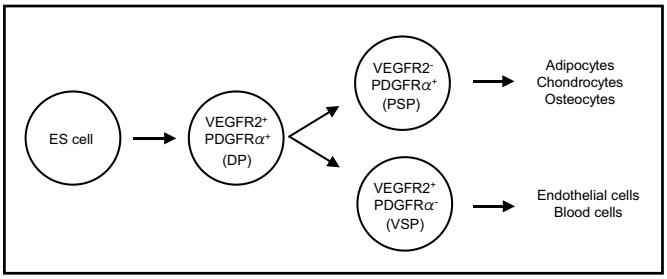

Fig.2 Differentiation pathway from ES to mesoderm-like cells

Three types of mesoderm-like cells can be generated from ES cells *in vitro*, PDGFRα^+VEGFR2$^+$ population (PDGFRα and VEGFR2 double positive population, DP), PDGFRα^+VEGFR2$^-$ population (PDGFRα single positive population, PSP) and PDGFRα^-VEGFR2$^+$ population (VEGFR2 single positive population, VSP). The DP is a common progenitor and can give rise to both the PSP and the VSP. The PSP and the VSP correspond to paraxial and lateral mesoderms, respectively.

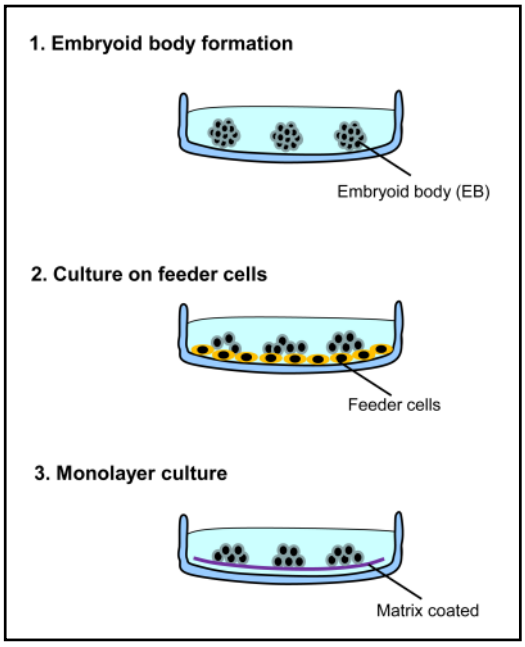

Fig.3 Methods of culture used for *in vitro* ES cell differentiation

The methods used for ES cell differentiation into various cell lineages are generally categorized into three types; (1) formation of embryo-like aggregates of ES cells; embryoid body (2) culture on feeder cells, such as OP9 stromal cells, and (3) culture on plates coated with a defined matrix such as collagen IV.

embryo, respectively. The analyses for gene expression in both populations also support the hypothesis that PSP and VSP correspond to paraxial and lateral mesoderms, respectively (Fig.2).

The knock-in/transgenic reporter strategies have been utilized to provide information on the various intermediate stages that occur during ES cell differentiation. Tracing the differentiated cell lineage by marker proteins also provides new information about the differentiation pathways. In fact, the existence of mesendoderm, which can give rise to both endoderm and mesoderm, was demonstrated by using the Goosecoid (Gsc) gene as a tracing marker[37, 38]. Brachyury (T), another lineage tracing marker, is expressed throughout the anterior-posterior region of the primitive streak and the notochord, and is associated with the appearance of mesodermal precursor cells. A 500 bp upstream promoter region of the T gene drives GFP expression in the middle portion of the primitive streak[39]. A combination of the markers, human CD4 targeted to the Foxa2 and T gene-drived GFP, could distinguish the cell populations corresponding to the anterior and posterior regions of the primitive streak, respectively[40].

Culture methods of cell differentiation

The culture methods of ES/iPS cell differentiation are one of factors important for the differentiation. The differentiation methods of ES/iPS cells are categorized into three types depending on the way of culturing: embryoid-body (EB) formation[41], co-culture with feeder cells[42], and simple monolayer culture on extracellular matrix-coated dishes (Fig.3). EB formation method is the most popular method. The three-dimensional cell aggregates are formed and they undergo a developmental process corresponding to the events of early embryogenesis. However, EB is composed of various cell types so that it complicates subsequent analysis. Furthermore, the aggregates of EB disturb investigators to control the differentiation because exogenous signals cannot reach to the inside of EB. Previous our study demonstrated that EB culture is less efficient in inducing mesendoderm cells expressing Goosecid, which is one of the markers for EGO, than the two-dimensional (2D) culture on collagen IV-coated dishes[37]. This result indicates an inherent limitation of EB system in guiding ES cell differentiation, as uncontrollable complexity is inevitably associated with three-dimensional architecture in EB. It is therefore difficult to direct ES/iPS cells into the cell of an interest by exogenous signals.

To overcome these problems, investigators have developed two-dimensional culture methods. Coculture with feeder cells allows the selective induction of the cells of an

interest. For examples, the co-culture with OP9 stromal cells provides the effective induction of blood cells from ES cells[42-44]. Similarly, PA6 stromal cells can support neurogenesis from ES cells[45]. OP9 stromal cell line is derived from op/op mutant mouse which carries the genetic mutation of M-CSF that is an essential factor for macrophage differentiation[46, 47]. The mutation results in inactivation of M-CSF so that OP9 culture enables the elimination of macrophages, which inhibits the proliferation of other hematopoietic cells. In addition, ST2 stromal cells, derived from bone marrow cells, can support osteoclast generation from hematopoietic cells, thus a sequential coculture of mouse ES cells with OP9 cells followed by ST2 cells could efficiently introduce osteoclasts[48]. Although some feeder cells have a potential to induce the cells of an interest selectively, the feeder culture system is technically complicated by the variable condition of feeder cells that affects viability and reproducible differentiation. A monolayer culture is simpler than feeder culture and provides us easily to control and observe the differentiation process, and to collect the differentiated cells. The culture dishes coated by an ECM, such as gelatin, collagen, fibronectin and Matrigel, are very useful for defined monolayer culture. Previous study has shown that collagen IV is suitable for directing ESC differentiation into mesoderm lineages including hematopoietic, endothelial and smooth muscle cells[30, 31]. Although each cell monolayer could be uniformly treated with the same culture conditions, lots of trials are needed to establish the defined culture conditions for the cell types of interest.

Another factor affecting the differentiation process of ES/iPS cells is the composition of the culture medium. Culture media containing fetal bovine serum are often difficult to reproduce because the combination of factors in serum varies among serum lots. In addition, undefined factors in serum may affect the differentiation from ES/iPS cells. To avoid these obstacles, serum-free conditions with supplements of chemically-defined factors should be developed[49]. The studies of mouse embryogenesis revealed that TGFβ, BMP, Wnt, Nodal and FGF families play important roles in early embryogenesis[16, 18]. BMP4 is an essential factor for mesodermal cells as well as primodial germ cells during embryogenesis. A serum-free medium containing BMP4 can support the induction of mesodermal lineages including blood and endothelial cells from ES cells on collagen IV-coated dishes[39]. Activin A that can act as a stimulator for nodal signaling, plays a role in mesoderm formation in gastrulation. In ES cell culture, Activin A also can induce mesendodermal differentiation from ES cells under chemically defined condition in the absence of serum[37, 38]. ESC culture with defined medium is acquired to apply ES/iPS cell-derived cells for cell therapies owing to keep safety of transplantation.

Mesenchymal stem cells and associated marker molecules

Since the concept of MSC was published, investigators have attempted to isolate MSCs directly from bone marrow (BM). Numerous cell surface molecules including CD105, CD73, MCAM (MUC18/CD146), CD29, CD44, CD49, CD106, CD166 (activated leukocyte cell adhesion molecule, ALCAM) and STRO-1 have been firstly identified as MSC markers[50-53]. CFU-F, a colony of MSC, can be enriched in STRO-1$^+$ population of BM[54]. The STRO-1^{bright}VCAM-1$^+$ subpopulation can differentiate into adipocytes, chondrocytes and osteocytes[54, 55]. CD146 and CD73 are popularly used as MSC markers[56-59]. Double positive cells isolated from BM can be differentiated into multiple mesenchyme lineages at a single cell level. Although any markers are not specific for MSCs, the single or combination of these markers enable us to enrich CFU-F colonies and to roughly purify MSC that can give rise to adipocytes, chondrocytes and osteocytes.

MSC is characterized and defined by cell morphology and differentiation capacity *in vitro*[5]. However, the origin and differentiation pathway of MSC remained to be elucidated. To address this question, the animal model is required for tracing MSCs and understanding the developmental process underlying molecular mechanisms. As mentioned above, no specific markers of MSC are identified and the expression of known markers is unclear during embryogenesis. We have shown that platelet-derived growth factor receptor α (PDGFRα) is a useful marker for MSC isolation during mouse development[60]. Using Sox1-Cre/Rosa-YFP mice, we demonstrated that the earliest MSC was developed from neuro-epithelial cells (NECs) that can form neural tube and subsequently give rise to neural lineage cells in mouse embryo. Furthermore, we found another type of PDGFRα^+ MSC that is not derived from NECs[60]. Interestingly, MSC originated from NECs continue to survive until neonates and quickly disappear after birth. These results indicate that although NEC-derived

MSC presents in mouse embryo, MSC that is originated from non-NECs become dominant during mouse development.

PDGFRα is useful for recognizing the adult type of MSCs as well as the embryonic type of MSCs. Morikawa et al. isolated PDGFRα^+Sca-1$^+$CD45-TER119$^-$ cells from adult mouse BM, which generated single cell-derived colonies at a high frequency and differentiated into hematopoietic niche cells, osteoblasts and adipocytes *in vivo*[61].

Generation of mesenchymal stem cells from ES/iPS cells

MSC is easily isolated from adult BM and other tissues such as fat[5, 62]. However, the isolations from adult tissues and the use of MSCs have several problems in term of clinical application. MSC isolated from elder people tend to exhibit the lower growth than that from young people. The cell number obtained from the MSC culture of elder people is so insufficient that the treatment with MSC is sometimes postponed. Second, the establishment of self-MSCs requires the periods to be expanded to reach to sufficient the cell number for the therapy. It is difficult in preparing the self-MSC immediately when it is necessary to use it. Preparing ES/iPS cell-derived MSCs gives a chance to help us solve these obstacles. Numerous investigators have reported the generation of mesenchymal-like cells from hESCs[63-66]. However, these cells were not fully characterized and classified in term of their differentiation and therapeutic potentials. Xu et al. reported derivation of fibroblast-like cells from human hESCs (H1 cell line)[65]. They immortalized hESC-derived mesenchymal cells by the forced expression of human teromerase reverse transpriptase (hTERT). The cells expressed MSC markers, including CD29, CD44, CD71 and CD90, and were capable of differentiating into an osteocytic lineage, but not chondrocytic and adipocytic lineages. Other groups also induced hESCs into fibroblastic/mesenchymal cells that express MSC markers such as CD90 and CD44 and can give rise to descendants of MSCs[66]. The MSC, which is fully characterized, were shown to be purified from *in vitro* ES/iPS cell culture by FACS using surface markers such as CD73 and CD105[64]. The gene expression analysis revealed that the MSCs isolated expressed the surface markers of adult-derived MSCs such as CD44 and STRO-1, and the other markers including DSC54, neuropilin 1, hepatocyte growth factor, forkhead box D1 and notch homolog 2[64].

The culture conditions that can selectively induce MSC from ES/iPS cells were also reported. The hESCs were cultivated with bFGF and platelet-derived growth factor (PDGF)-AB under a feeder-free condition, and then the CD105$^+$CD24$^-$ population was isolated by FACS[63]. This population was found to differentiate into adipocytic, chondrocytic and osteocytic lineages. The cultivation of mouse iPS cell-derived EBs with TGF-β1 and retinoic acid (RA) produced MSC-like cells that could differentiate into osteoblasts[67]. Similarly, the hiPSC-derived MSCs were generated under the condition with bFGF, PDGF-AB and epidermal growth factor (EGF) following by isolating the CD105$^+$CD24$^-$ population by FACS[68]. In summary, although MSCs and MSC-like fibroblastic have been isolated and characterized from *in vitro* ES/iPS cell culture, the differentiation pathways and their intermediates still remained unclear.

We searched for the MSC differentiation pathway in mouse ESCs and found a novel pathway in which MSC was generated from neuroepithelial cells[60]. As mentioned above, PDGFRα^+ mesodermal cells are generated in the absence of Retinoic acid (RA) under a conventional condition containing serum. The treatment with RA can significantly induce ESC differentiation into the neuroepithelial lineage by suppressing mesodermal and endodermal differentiations. The generation of PDGFRα^+ mesodermal cells was suppressed by RA treatment at early stage (~day 4) and its proportion was subsequently peaked at the late-stage, day 9. This Day 9 PDGFRα^+ cell exhibited a fibroblast-like morphology and expressed MSC markers such as OB-cadherin (cadherin 11) and PGDFRβ . The Day 9 PDGFRα^+ cell underwent sustained proliferation *in vitro* and kept the potential to differentiate into three principle mesenchymal lineages including adipocytes, chondrocytes and osteocytes even after 30 days culture. These results indicated Day 9 PDGFRα^+ cell was satisfied with MSC definition. RA treatment also enhanced the expression of Sox 1, which is a marker of neuroepithlium in mouse development. To investigate the differentiation pathway, we took advantage of the Sox1$^{gfp/+}$ ES cell line carrying *a gfp* cDNA inserted in the allele of Sox1 and examined the fate of the GFP-positive population in *in vitro* ES cell culture. As GFP expression can mirror Sox 1 expression in this ES cell line, the fate of Sox 1+ cells was easily traced during ES cell differentiation. The GFP$^+$ population, but not GFP$^-$, generated from Sox1$^{gfp/+}$ ES cell line could differentiate into

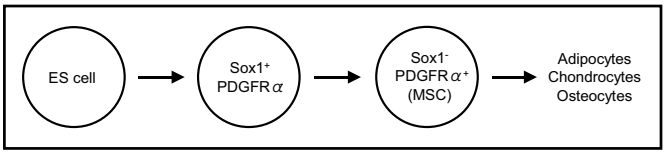

Fig.4 A novel pathway of MSC differentiation
Neuroepithelial cells (NEs) is one of precursors of MSC. Sox1$^+$ NEs give rise to PDGFRα^+ mesenchymal stem cells.

PDGFRα^+ cells and subsequently gave rise to adipocytes. Our results have demonstrated that Sox1$^+$PDGFRα^- differentiate into MSC via Sox1$^-$PDGFRα^+ stage in *in vitro* ES cell culture[60]. To explore the *in vivo* differentiation pathway of MSCs derived from Sox1$^+$ neuroepithelium, we generated Sox1-Cre mice and mated with Rosa26-STOP-EYFP. Sox1-Cre mice carried Cre recombinase cDNA inserted into the Sox1 allele. In mated mice, Cre expression is regulated under Sox1 promoter and induced EYFP expression driven by constitutive promoter of Rosa26. Thus, the fate of Sox1$^+$ cells is easily traced as YFP$^+$ cells in mouse development. In E14.5 embryos from Sox1-Cre/EYFP mice, the PDGFRα^+ population derived from Sox1$^+$ neuroepithelial cells were present in embryo trunks and contains MSCs.

Taken together, we demonstrated a novel differentiation pathway in which MSCs are originated from Sox1$^+$PDGFRα^- neuroepithelial cells both *in vivo* and *in vitro* (Fig.4)[60]. Other group confirm the evidence that adult MSCs partially originate from the neural crest, a progenies of neuroepithlial cells, by tracing PDGFRα^+Sca-1$^+$CD45$^-$TER119$^-$ cells in adult mouse BM[69].

Perspective～A risk of tumor formation～

Although human ES cell is expected for regenerative medicine, both the ethical problems and the immunological rejection are the main obstacles for the application of human ES cells as a source for clinical treatment. The iPS cells can overcome these obstacles because self-pluripotent stem cells (self-iPS cells) can be easily generated from somatic cells such as blood cells. However, the transplantation of ES/iPS cell-derived cells is attended by the risk of tumor formation. The insufficient purification of transplanted cells induces contamination of undifferentiated ES/iPS cells that may form teratoma after transplantation. It is a critical step for clinical application to develop and establish robust and efficient methods of differentiation, which can completely exclude the tumorigenic undifferentiated cells. To achieve that, more fundamental studies are required to dissect the intermediate processes producing the differentiated cells of interest and to explore unknown cell surface markers for the purification of the target cells.

Another strategy to avoid the contamination of undifferentaited ES/iPS cells is to develop the method for the differentiation and the maintenance of intermadiates such as somatic stem cells. If ES/iPS cell-derived somatic stem cells can be maintained *in vitro*, it expects to remove the contamination of undifferentiated ES/iPS cells and to help our understanding of the molecular mechanisms of self-renewal and multipotency of somatic stem cells. Therefore, we can avoid the risk of tumor formation with contaminated pluripotent stem cells and take advantage of the induced somatic stem cells for clinical applications.

Acknowledgments

This study was supported in part by grants from the Ministry of health, Labor, and Welfare of Japan and Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency.

Source of Funding and Conflict of Interest

None

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