Biology of Mesenchymal Stem Cells

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Abstract: Mesenchymal stem cells (MSC) are derived from mesodermal precursor and are committed towards mesenchymal differentiation. They are scattered all over the organism, situated in bone, cartilage, adipose tissue and accompany organs for tissue regeneration and structural and functional support. MSC populations are not homogenous, their signature is variable according to their localization. A process called "epithelial mesenchymal transition" is fundamental for the development of mesoderm. Epithelial-mesenchymal interactions specify MSC and this may influence their regeneration potential. Multipotent adult MSC are used for research in tissue regeneration and engineering. Crude mixtures of bone marrow-derived MSC are clinically applied for tissue healing, but complex transplantable tissue engineered constructs are still under development. The role and regeneration potential of MSC in inflammation and ageing organisms remains to be characterized. The establishment of reprogrammed homogenous MSC cultures of high plasticity might allow developing these cells towards multiple cell-based therapeutic strategies. Many applications can be envisioned, e.g. regeneration of bone, cartilage and tendon or engineering of beta cells and neurons. Since homogenous MSC with high plasticity represent a promising tool for the treatment of many diseases, research in this area of adult stem cells should be supported with high priority.

STEM CELLS – FROM EMBRYONIC TO SOMATIC STEM CELLS

Embryonic stem (ES) cells are localised in the inner cell mass of blastocysts. They are capable of indefinite self renewal and, being pluripotent, can develop into any cell of an organism. ES cell lines have been described since almost a decade by two independent groups [1, 2]. The accessibility and usage of ES cell chromatin is tightly regulated in that they can activate the relevant genes for self-renewal and proliferation and silence genes relevant to differentiation and commitment [3-5]. Polycomb proteins are important in regulating such nuclear events. Polycomb protein complexes are able to bind to and to methylate promoter regions which are relevant for fate decision and differentiation [6, 7]. Polycomb group complexes (PRC) I and II act in a coordinate fashion in that PRC II methylates histones in critical regions which then attracts PRC I complexes to silence the respective genes [8]. There is also increasing evidence that regulatory micro RNAs may contribute to fate decision and differentiation versus stemness maintenance in various stem cell populations [9]. In addition to repressors of differentiation at the genomic level there are morphogens and their inhibitors (e.g. BMP, Hedgehog) at the proteomic level which are expressed to regulate differentiation induction and spontaneous differentiation [10, 11]. Their role during development is also important in defining boundaries of developing tissues and building gradients for patterning [12, 13]. Retinoic acid appears to be one of several important morphogens during ES cell commitment and differentiation [14]. Downstream of morphogen stimulation differential activation of kinase cascades like the mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK), c-Jun amino-terminal kinase (JNK), and p38MAPK regulates ES cell commitment. The activation of the ERK pathway, which inhibits self renewal of ES cells and influences mesodermal commitment of ES cells, is also of relevance for the development of adult mesenchymal stem cells (MSC) [15]. The pathway of differentiation of ES cells into committed MSC is not yet unravelled although a recent report described the establishment of stable ES-derived clones with MSC-like attitudes [16].

HALLMARKS OF EMBRYONIC STEM CELLS AND REPROGRAMMING ADULT STEM CELLS

The transcription factor Oct4 is a key regulator of pluripotency in ES cells and is rapidly downregulated with ongoing commitment of ES cells towards the different germ layers. Downstream Oct4 a considerable number of genes and secondary regulators are modulated which are relevant in terms of differentiation and commitment, e.g. Sox2, Nanog (enhanced) and Cdx2, BMP4, Dlx5 (inhibited) [17]. Recent publications report on reprogramming of adult somatic cells into a pluripotent state towards ES stem cells by using somatic-cell nuclear transfer and cell fusion with ES cells. Also retroviral transduction of Oct4, Sox2, c-myc and Klf4 were described to achieve this goal [17-21]. Nanog, first discussed to be another candidate appears to be dispensable for somatic pluripotency but is required for the formation of germ cells [22]. Overall a set of such transcription factors has been discussed to be responsible for stem cell reprogramming but obviously a single factor of all candidates can not yet be identified. The interdependence and reversibility of reactivation of transcription factors mediating pluripotency like Oct4 versus the commitment towards a distinct germ layer remains to be dissected in order to gain insight into the

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molecular mechanisms of reversibility of commitment and consecutive plasticity in stem cells.

ADULT MESENCHYMAL STEM CELLS

The evolution of mesoderm and muscle is central in metazoan evolution, where the majority of organisms are triploblasts, which exhibit three discrete germ layers [23]. The vertebrate musculoskeletal system develops from the mesoderm [24]. Basal transcription factors orchestrate the development of various parts of the musculoskeletal system. A distinct pattern of Hox genes is linked to the development of the vertebrae [25], muscle development requires expression of myoD and concerted signalling of Hedgehog and FGF gene products [26], and synovial joints and articular cartilage develop from interzone cells expressing GDF-5, Wnt-14 and CD44 [27]. Mesenchymal cells not only give rise to classical mesenchymal organs and tissues but also connective tissue accompanies any organ structure in the organism. The physiological tasks of these cells remain to be characterized but it is obvious that they support differentiation maintenance, assist in injury healing and give rise to fibrous substitutes, e.g. scars, where the original tissue can not be replaced by restitutio ad integrum. Interestingly a recent report described the idea of the production of "matrix super-highways" by MSC to support organ growth *in vivo* and *in vitro* [28].

Accompanying all mesenchymal tissues multipotent adult MSC harbouring mesodermal and mesenchymal commitment are spread all over the organism [29]. Their genomic signatures may however be variable, depending on their localisation, and their physiological roles in the organism may also vary considerably. Multiple sources of MSC (e.g. bone marrow stroma, adipose tissue, bone chips, synovia) have been characterized more closely, which all display similar differentiation capacity when tested *in vitro* and *in vivo* and they are developed towards clinical use for regenerative medicine [30]. Their capacity to give rise to various mesenchymal tissues and also to modulate host immune responses provokes expectations even in terms of allogeneic application settings of tissue engineering and regenerative medicine (see also below) [31-33].

EPITHELIAL MESENCHYMAL TRANSITION STATES

Epithelial mesenchymal transition (EMT) occurs during embryogenesis, indicating that mesenchymal cells do not only develop from mesoderm but also from epithelial endodermal cells. This process appears to be an indispensable mechanism during morphogenesis since organ formation needs mesenchymal cells. There are however also reports about the reverse process, mesenchymal epithelial transition. The EMT process of epithelial sheets can be reversible or irreversible. The process is tightly involved in structure development, e.g. neural structures, craniofacial structures and vertebrae [34]. The phenomenon has also been extensively described in ES cell cultures without feeder layers, in the context of angiogenesis, where endothelial and mesenchymal cells together form vessels, and it has also been impressively demonstrated by the development of epithelial structures from mesenchymal precursors in the skin [35-38]. A population of pancreatic apparently epithelial mesenchymal transient MSC like cells has been discussed and described in extenso on the background of the possibility of regenerating endocrine beta cells. Such populations are associated with pancreatic islets, can be easily obtained and differentiated at least in part towards endocrine phenotypes [39, 40]. Experiments using very similar fibroblast like cells from skin demonstrated also mesenchymal endodermal transition in that hepatocytes could be developed from this population [41]. In summary there are MSC like populations which are different from classical MSC populations in that they display mixed phenotypes in terms of germ layers. Transition between epithelial and mesenchymal phenotypes and vice versa appears to be common. Their exact physiological tasks during adult life remain to be characterized and their therapeutic potential is also not yet ultimately validated (see also below).

MESENCHYMAL STEM CELLS AND THEIR NICHE

The present view of a stem cell in an adult organism is that it is a rather quiescent cell, which divides rarely and which resides in a protected niche, constituted by somatic non-stem cells [42]. Such niches can be simple or complex, providing homes for either one or more defined subpopulations of stem cells of even different origin (Fig. **1**). Cell division in stem cells can be symmetric or asymmetric. While the former events control the stem cell pool of a rather homogenous stem cell population, the latter gives rise to an already committed population frequently named as "transient amplifying compartment". This compartment is probably identical with the populations we are working with if we establish primary mesenchymal stem cell culture [30, 43]. The very niche of a mesenchymal stem cell has not yet been identified, but MSC and their osteoblast-like offspring constitute the hematopoetic stem cell niche [44]. It may also be discussed, if MSC of various origins reside in identically composed niches or if the niches are as variable as MSC subpopulations and localisations. Recent publications suggest a perivascular nature of MSC niches. Based on surface marker characteristics of MSC-like expression of CD146, Stro-1, Sca-1 and Thy-1 and the lack of expression of CD11b, CD34, CD45, CD117 and CD31 several authors have identified niche like structures that require further characterization. Overall surface markers like CD146, the combination of Stro-1 and CD106 and CD73 are suggested by several authors to be most characteristic to phenotype MSC populations (Table **1**) [45, 46].

MSC RECRUITMENT

MSC can be recruited to sites of injury or tissue repair by several proteins and compounds. They are able to migrate and a small number circulates in the peripheral blood. Thus both systemic and local recruitment to sites of repair have been described like in the situation of myocardial infarction [47-52] and in bone healing [53-55]. Several mediators of MSC migration have been described, e.g. BMP2 and 4 and PDGF-bb and also members of the CCN family like CYR61 and WISP3 [56, 57]. Synovial fluid after injury is also capable of stimulating MSC migration [58]. The invasive capacity of MSC to cross endothelial barriers is controlled by the balanced expression of matrix metalloproteinases and their inhibitors as has been reported for MMP-2, MT1-MMP and TIMP-2 [59]. Overall there is increasing evidence that MSC can be recruited from relatively far distance by chemotaxis and migration in situations of tissue repair and healing.

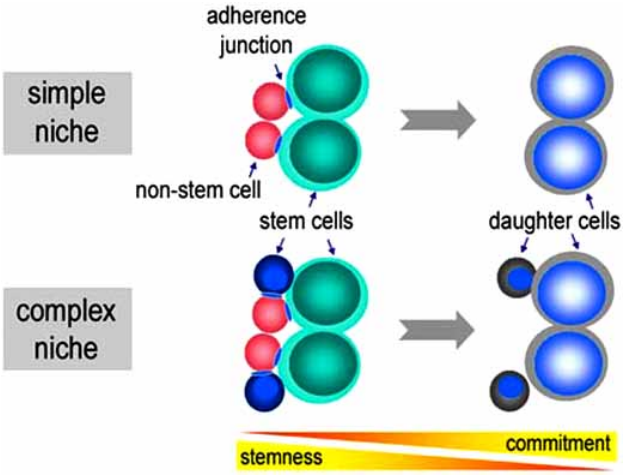

Fig. (1). Models of stem cell niches. Stem cell niches are comprised of stem cells and non-stem cells, which are connected by adherence junctions. While simple niches contain only one defined stem cell subpopulation, more than one stem cell subpopulation resides in complex niches. In both niches, stem cells give rise to daughter cells. Thereby, stemness declines with advancing commitment of these cells [45,46].

Table 1. Surface Antigens of Mesenchymal Stem Cells

Surface Antigen	Name
Positive	
ALP	Alkaline phosphatase
CD13	Aminopeptidase N
CD29	Integrin β1
CD44	Hyaluronate receptor
CD49a	Integrin α1
CD63	Melanoma 1 antigen
CD73	5' nucleotidase
CD90	Thy-1 antigen
CD105	Endoglin
CD106	Vascular cell adhesion molecule 1
CD140b	Platelet-derived growth factor receptor β
CD146	Melanoma cell adhesion molecule
Stro-1	Stromal precursor cell marker 1
Negative	
CD11b	Integrin α M
CD31	platelet/endothelial cell adhesion molecule
CD34	Hematopoietic progenitor cell antigen CD34
CD45	Leukocyte common antigen precursor
CD117	Mast/stem cell growth factor receptor precursor

Surface markers, which characterize MSC populations, are listed as "positive". Surface Markers, which are absent in MSC populations are listed as "negative" [42, 45, 46].

MSC DIFFERENTIATION POTENTIAL

Primary MSC from various locations can be differentiated towards the osteogenic, chondrogenic and adipogenic pathways of differentiation *in vitro* and *in vivo* [30]. The *in vitro* procedures of differentiation are now more or less standardized and will not be reviewed here. However if studied in more detail, various populations are distinct from each other, e.g. in terms of their response to osteogenic stimuli and their genomic signature [60]. When cultured in collagen type 1 and subjected to cyclic strain they can also produce ligament like extracellular matrix components indicating their differentiation towards tenocytes [61]. In the special context of this review it is important to realize that equally multipotent populations of MSC have been isolated and are presently characterized from synovial tissues like fibrous and adipose synovium and that their chondrogenic potential is reported to be even superior compared to bone marrow derived MSC [62-64].

When immortalized by telomerase transduction the respective cells (hMSC-TERT) can also display an endothelial cell like phenotype [65]. Myogenic differentiation can be obtained by using muscle derived satellite cells, but was also recently reported to be initiated from vascular pericytes, possibly indicating that this pathway of differentiation can only be initiated by a discrete subset of MSC [66, 67].

There is an ongoing discussion as to whether MSC can acquire pluripotency or at least extend their panel of multipotency to be used as substitutes for ES cells for cell based therapeutic strategies. So far it is not clear, if there is a natural MSC-like stem cell with sufficient "stem cell plasticity", which can give rise to multiple tissues, being nearly or really pluripotent without prior reprogramming (see also below), as it was discussed in earlier work where MSC were injected into blastocysts [68]. Thus the intrinsic differentiation capacity of MSC beyond osteogenesis, chondrogenesis, adipogenesis and tenogenesis is everything but clear. Reports about the *in vitro* generation of e.g. neuron like, endothelial-like, hepatocyte-like cells from MSC have to be critically reviewed according to the question if these MSC only support local resident cells or really undergo differentiation. In case of hepatocytes there are several reports of hepatogenic differentiation from MSC, others vice versa reported that MSC with osteogenic capacity developed from liver cell precursors [69, 70]. Extremely contradictory results report also that MSC secrete hepatocyte growth factor and are capable of stimulating liver regeneration, or have even no influence at all on liver regeneration [70-73] Multiple approaches to obtain insulin-producing cells from primary MSC under certain culture conditions and through transduction of cells with key transcription factors have also been reported. This yielded variable cell phenotypes developed from MSC expressing insulin and C-peptide, which were partially glucose sensitive and ameliorated diabetes in animals. Still this therapeutic potential of MSC in a human setting remains an open question, especially if and to what extend reprogramming is required to achieve complete endocrine differentiation and glucose response [40].

Altogether, MSC are considered multipotent stem cells, although their pattern of differentiation pathways may vary

due to their "stemness", their degree of commitment and their genomic signature. The term "mesenchymal stem cell" is uniformly used for populations of various origin, genomic signature, stemness and plasticity and in consequence of variable differentiation capacity. Moreover, the populations characterized are either clonal immortalized populations and therefore somewhat artificial, or they represent a non-homogenous transient amplifying pool which is already different from the stem cell that gave rise to this population by asymmetric cell division. Since primary MSC from bone marrow stroma already comprise variably clonogenic subpopulations, acceptably homogenous populations can only be obtained by strict maintenance of differentiation status already during isolation and by sorting for the above discussed panel of surface markers [45]. The very stem cell however can only be characterized in its natural environment of the niche, which remains to be done. Whether there are stem cells in adult tissues, which are in a stage of very early mesodermal commitment or in various germ layer transition states that can be easily reprogrammed towards pluripotency, remains also to be shown. The more we learn about the characterization of the variable stages and pathways of differentiation of such MSC populations the more we can envision that reprogramming of such cells can become a valid tool to obtain versatile populations for cell-based therapeutic strategies.

MSC REJUVENATION VERSUS AGING AND TUMOURIGENESIS

MSC from young individuals or from fetal sources are different from adult MSC [74, 75] in that they express stemness related markers and display pronounced proliferation and differentiation capacity. Although there are contradictory reports about MSC from aged donors the number of clonogenic MSC appears to decline with ageing [76]. Ageing is associated with several hallmarks like damage accumulation, telomere shortening, loss of stemness related marker genes, impaired lamin functions and failure of DNA processing enzymes including DNA repair enzymes [77]. Telomerase reactivation or transduction is associated with features of "rejuvenation" but at the same time when applied to cells, which already accumulated damage, harbours the danger of tumour promotion. As recently shown, even primary prostate epithelial cells, when transduced with telomerase, expressed Oct4 and several multilineage markers, indicating that they obtain multipotent differentiation capacity [78]. Telomerase-immortalized MSC were described by Kassem and coworkers. They can be differentiated towards various differentiation pathways but can also produce tumours in nude mice after sequential acquisition of stepwise defined mutations if kept under high proliferative pressure [65, 79, 80]. MSC when cultured ex vivo show signs of genotoxic stress but can be protected by enhancing antioxidative systems in culture [81-83]. This indicates that ex-vivo cultured MSC may accumulate cell damage and may develop towards replicative senescence. The latter represents a fail safe program of cells to avoid both apoptosis and tumour development. In replicative senescence cells stop dividing and their specific functions may be impaired, although they are still part of an organized tissue volume [77, 84]. If an overriding proliferation stimulus like telomerase overexpression is set in a senescent cell, this cell is at high risk to cause tumour development as the reactivation of telomerase expression has been shown to represent a hallmark in certain pathways of tumourigenesis [85].

Cell-based therapeutic strategies are developed for regenerative medicine in various situations. However the most frequent situation where enhancement of regeneration is needed occur in the elderly and in situations of pathology. Addressing aged cells and impaired cells for rejuvenation and proliferation has to be carefully evaluated in terms of risks and benefits. Moreover, strategies to selectively target non-senescent cells in individuals should be developed for the sake of security in cell-based therapies. Besides the characterization of intrinsic MSC attitudes associated with ageing also dissection of the humoral environment appears to be extremely relevant since at least some rejuvenation of aged progenitor cells can be achieved by exposing them to a "young" environment [86].

MSC AND INFLAMMATION

Immunomodulation

MSC display low immunogenicity and moreover show active immunosuppressive effects on T-cells and dendritic cells [87]. MSC can induce divisional arrest anergy in T cells and by stem cell production of soluble immunomodulatory factors, including interleukin-10, transforming growth factor-beta, prostaglandin E2, and hepatocyte growth factor can modulate immunosuppression. Moreover, they can prolong skin allograft survival and may decrease graft-versus-host disease (GVHD) in the setting of allogeneic transplantation [88]. It was also shown in the murine system that MSC can ameliorate autoimmune encephalitis and thus have immunosuppressive potential [89]. Thus the potential of MSC derived cell lines to serve as a versatile cell population in the setting of allogenic transplantation or for supportive treatment of autoimmune diseases should be discussed and evaluate

In osteoarthritis and rheumatoid arthritis MSC may be attracted to the sites of degeneration and inflammation. The local populations are situated in the synovia and for example in Hoffa´s fat pad in the knee joint [90]. Their role and relevance in the process of inflammation and repair is probably substantial but little is known about their function under the influence of uncontrolled proinflammatory stimuli and their impairment by side effects of inflammation like reactive oxygen species spillover from inflammatory sites. This is of special interest since there is evidence that cellular ageing may be substantially propagated by inflammation, and senescent cell phenotypes show symptoms of activation of proinflammatory genes. For this reason "natural" ageing has also been described as a consequence of chronic inflammation. In fact the term "Inflame-Aging" has been coined based on experimental evidence that has been demonstrated in the context of characterizing the molecular events in the classical Werner´s progeria syndrome [91, 92]. MSC *in vivo* recruitment to the synovial surface has recently been demonstrated in patients with osteoarthritis [93]. However it is essentiell to show efficient function and survival of MSC and MSC based tissue constructs in inflamed tissues for a sane rational to apply cell based therapies in this situation. If anti-inflammatory mechanisms are not provided at the same time, one could argue that delivery of MSC into inflamed joints

might even contribute to an exaggerated pannus forming and joint eroding reaction caused by a population of fibroblast-like synoviocytes. The latter are discussed to represent MSC which by proinflammatory stimuli have been arrested in their differentiation and are even "misused" for promotion of disease progression [94]. This indicates that in a setting of regenerative strategies one would urgently have to control for the underlying pathology to force the precursor cell population applied towards healing and regeneration and restrain it from propagating underlying pathology. This potential of cell-based strategies as a double-edged sword has to be discussed more intensely.

SUMMARY AND CONCLUSIONS

Mesenchymal stem cells are derived from mesodermal precursor and are committed towards mesenchymal differentiation. They are scattered all over the organism, form classical mesenchymal tissues like bone, cartilage, adipose tissue and muscle, but also accompany organs to support their function. The MSC preparations one can easily obtain from bone marrow, adipose tissue and other sources are not homogenous populations, they do even show considerable variability in terms of their signatures according to their localisation. A process called "epithelial mesenchymal transition" appears to be indispensable during development and is involved in the development of structures like craniofacial structures and vertebrae, but also in regeneration procedures later in life. This process demonstrates that MSC-like cells can also develop from epithelial cells. The molecular dissection of various MSC signatures and function in adult life is just at the beginning. Multipotent adult MSC are developed as tools for tissue regeneration and engineering [30, 76]. Crude mixtures of bone marrow-derived MSC have been clinically applied for tissue healing, but tissue engineering ex vivo has not yet produced high quality constructs, which can be used for routine transplantation procedures [95]. MSC in culture rather rapidly undergo commitment and develop replicative senescence after approx. 6-15 passages. If we succeed in establishing reprogrammed homogenous MSC cultures of high plasticity, we might be able to further develop these cells towards multiple cell-based applications. The list of potential applications is long and comprises regeneration (e.g. of bone, cartilage and tendon), engineering (e.g. of beta cells and neurons), immunosuppressive support in autoimmunity and transplantation, and an almost indefinite battery of engineered cells, which can be transplanted for local drug delivery on request (e.g. anti-inflammatory molecules like IL1RA governed by an inflammation driven promoter). Since homogenous MSC with high plasticity represent a promising tool for the treatment of many diseases, research in this area of adult stem cells should be supported with high priority.

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