1

The Biology of Human Mesenchymal Stem Cells

Claire Westwood and Mark O. Clements

Introduction

Mesenchymal stem cells (MSCs) are a population of progenitor cells derived from the bone marrow which are capable of differentiating into mesenchymal tissues. There is growing interest in development of MSCs as a therapeutic agent for the treatment of a wide range of diseases, as well as providing a model system to investigate human mesenchymal development.[1,2] This review will focus on the historical evidence for the existence of MSC, as well as recent progress in our basic understanding of these cells.

Historical Perspective

The first evidence supporting the existence of bone marrow cells with the potential to form various mesenchymal tissues arose from experiments performed during the 1950s and 1960s. The osteogenic potential of bone marrow was demonstrated by animal host transplantation of whole bone

STEM CELL REPAIR AND REGENERATION - Volume 3
© Imperial College Press
http://www.worldscibooks.com/lifesci/p535.html

marrow samples into various body sites. Histological analysis of the transplantation sites revealed extensive formation of tissues resembling bone and marrow stroma, which could also support haematopoiesis from invading host haematopoietic stem cells.[3,4] Similar experiments involved the use of diffusion chambers, which consist of semi-permeable membranes separated by a plastic ring that do not allow passage of donor or host cells. Transplantation of chambers, containing either fragments or cell suspensions of bone marrow, resulted in formation of osteogenic tissue within the chamber after a month.[5] This indicated that, rather than the donor material attracting osteogenic host cells, there existed within the transplanted marrow stroma a progenitor cell with the potential to form bone.

Friedenstein and co-workers later showed that the osteogenic potential of bone marrow was a feature of a small population of cells, termed fibroblast colony forming cells (FCFC) or colony forming units-fibroblastic (CFU-F). The *in vitro* culture of guinea pig bone marrow at low cell densities in serum-containing media gave rise to fibroblastic colonies derived from single cells.[6,7] CFU-F had a high proliferative capacity *in vitro*, but were in a resting state *in vivo*.[7] Studies of the human counterpart of CFU-Fs were in agreement with this observation.[8] The transplantation of diffusion chambers containing cultured guinea pig CFU-F into the intraperitoneal cavity of host animals resulted in the formation of bone, which did not occur upon transplantation of spleen-derived fibroblasts.[6] This work was confirmed and extended by others, by showing extensive formation of bone, cartilage and fibrous tissue within diffusion chambers inoculated with marrow fibroblast cultures.[9]

This led to the concept of the existence of an undifferentiated stromal cell precursor in the bone marrow, capable of forming both bone and cartilage; however the presence of committed progenitor cells, each giving rise to a different cell type, could not be ruled out. To address this issue, single fibroblastic clones were transplanted under the renal capsule. About 15% of transplanted colonies produced a bone marrow organ, consisting of osteogenic tissue, typical adipose cells and marrow stromal cells.[10] Thus, colonies derived from a single cell were capable of giving rise to multiple cell lineages.

The concept of a multipotent stem cell of stromal origin residing in bone marrow was formally presented by Owen in 1978.[11] It was suggested that the differentiated cell types residing in marrow stroma may derive from a single common progenitor, or stem cell, analogous to the haematopoietic system within the marrow.[11] A more detailed model was

STEM CELL REPAIR AND REGENERATION - Volume 3
© Imperial College Press
http://www.worldscibooks.com/lifesci/p535.html

later proposed, in which stem cells, committed progenitors and mature cells were all present in marrow stroma, with reticular, fibroblastic, adipocytic and osteogenic being their possible differentiated cell types.[12,13] Caplan expanded this theory to include all mesenchymal lineages, namely myocytes, chondrocytes, tenocytes, osteocytes, adipocytes and stromal and dermal fibroblasts, since they are all derived from the same embryonic origins. He was the first to use the term mesenchymal stem cell, however there was no experimental evidence at the time to support this theory.[14]

The mulitpotential nature of MSC was in part demonstrated by the early studies described above. Further evidence was provided from differentiation of conditionally immortalised mouse MSC clones, one of which had the ability to form four mesenchymal phenotypes: chondrocytes, osteoblasts, adipocytes and haematopoiesis-supportive cells.[15] However, final conclusive evidence that clonal populations of human MSCs were capable of differentiating *in vitro* to osteocytes, chondrocytes and adipocytes was provided by Pittenger *et al.*[16] This study also defined a set of cell surface markers expressed on MSCs and estimated their frequency in the bone marrow to be 0.001%–0.01% of nucleated cells.

Differentiation Potential of Bone Marrow-Derived MSCs

Adipocytes, osteoblasts, chondrocytes and haematopoiesis-supportive stroma

As described earlier, it is well established that clonal MSCs have the capacity to differentiate *in vitro* to adipocytes, osteoblasts, chondrocytes, and also to haematopoiesis-supporting stromal cells, in the case of murine MSCs.[15,16] Robust and specific hormonal regimes capable of inducing the *in vitro* differentiation of MSCs into adipocytes, osteocytes and chondrocytes are well established.[16,17]

Tenocytes

MSCs from rabbit bone marrow were shown to differentiate into tenocytes, capable of replacing severed tendon in an animal model,[18] but this lineage is little studied, and *in vitro* differentiation of clonal human MSC populations to tenocytes has not been reported.

STEM CELL REPAIR AND REGENERATION - Volume 3
© Imperial College Press
http://www.worldscibooks.com/lifesci/p535.html

Cardiac and skeletal muscle cells

There is growing interest in the therapeutic potential of MSCs for cardiac and skeletal muscle repair. The differentiation of MSCs to cardiomyocytes using animal models has recently been reviewed by Zimmet and Hare,[19] and so will not be discussed here. In contrast, there is only limited evidence of *in vitro* differentiation of MSCs into cardiomyocytes. Spontaneously beating cardiomyocyte-like cells have been generated by treating immortalised murine MSC clones with 5-azacytidine.[20] However, as differentiation of these clones to other lineages was not assessed, it cannot be ruled out that these cells were unipotential progenitors rather than multipotential MSCs. Similar results were obtained when human MSCs were treated with 5-azacytidine, but non-clonal MSC populations were used.[21] Thus, there is some evidence of differentiation towards the myogenic lineage by cells that co-purify with MSCs, but further work using clonal populations and demonstrating multilineage potential is required to show that this is a property of MSCs rather than more restricted progenitor cells.

Hepatocytes

An early study reported the upregulation of hepatocyte markers when human MSCs were treated with hepatocyte growth factor, but no functional studies were performed.[22] More recently, co-culture of MSC with hepatocytes using a transwell format was reported to induce the differentiation of human MSCs into cells with hepatocyte function.[23] However, both of these studies used non-clonal MSCs therefore the presence of contaminating progenitors cannot be ruled out.

Neural cells

Several studies using rodent and human MSCs claimed to have induced *in vitro* neural differentiation using β-mercaptoethanol (β-ME) or dimethyl sulphoxide (DMSO) to initiate the process. This protocol was pioneered by Woodbury and co-workers who described the expression of neurological markers and outgrowth of neuronal processes after treatment of human or rat MSCs.[24] Similar studies reported the generation of neurons with electrophysiological properties,[25] or with characteristics similar to neurons produced after co-culture with Schwann cells.[26] However, it was recently reported that β-ME or DMSO/BHA treatment causes stress-induced

STEM CELL REPAIR AND REGENERATION - Volume 3
© Imperial College Press
http://www.worldscibooks.com/lifesci/p535.html

depolymerisation of the actin cytoskeleton and retraction of the cytoplasm in numerous cell types, thus the "neural" morphology observed was a result of stress rather than differentiation.[27,28] Moreover, no increase in neural marker expression was seen by Western blot analysis, and therefore it was concluded that the increase in immunohistochemical staining for neural markers was due to higher antigen density associated with smaller cell size.

Other *in vitro* studies have utilised growth factors known to promote neural differentiation of ESCs and neural stem cells. Incubation of human or mouse bone marrow stromal cells with differentiation medium containing such factors, or co-culture of labelled murine MSCs with rat midbrain cell suspensions, resulted in a small percentage of cells with morphologies similar to immature neurons or glial cells and occasional expression of neural markers,[29] however, cell fusion was not discounted from the co-culture experiments. In another study, Hermann and co-workers used suspension culture of non-clonal hMSCs followed by incubation with different neural growth factors.[30] Cells with various phenotypic and functional properties reminiscent of neural cells were obtained, including: dopamine-releasing cells expressing some neural markers and cells with phenotypic characteristics of astrocytes, oligodendrocytes and rare immature neuron-like cells. A proportion of cells treated with a specific growth factor had voltage-dependent electrophysiological outputs in patch-clamp experiments typical of glial cells.

In vivo differentiation potential

Several studies have reported engraftment of labelled murine MSCs into irradiated mice using systemic infusion. This included differentiation and engraftment into mesodermal tissues (marrow, bone and cartilage[31]) and signs of osteoblast differentiation in an osteogenesis imperfecta mouse model.[32] In a more recent study, GFP-tagged, phenotypically characterised murine MSCs were intravenously infused into minimally irradiated mice, and were found after one month mainly in the lungs, liver and kidney, but also in the muscle, heart, brain and spleen.[33] Immunohistochemical assessment revealed that MSCs differentiated into lung epithelial cells, hepatocytes, renal tubular-like cells and myofibroblasts, albeit at a low frequency. *In vivo* differentiation of human MSCs has been studied by transplantion *in utero* in sheep where they were found to engraft and survived in multiple non-haematopoietic tissues for up to 13 months.[34]

© Imperial College Press
http://www.worldscibooks.com/lifesci/p535.html

Furthermore, MSCs were found to differentiate into cells with morphological and phenotypic properties of chondrocytes, adipocytes, bone marrow stromal cells, thymic epithelial cells, cardiomyocytes and skeletal myocytes. However, the human MSCs used for this study were not clonal, and cell fusion was not investigated.

MSCs are also reported to differentiate into neural cells *in vivo*. Kopen *et al.* injected BrdUrd-labelled murine MSCs into neonatal mouse brains.[35] Cells double-labelled for BrdUrd and GFAP, an astrocyte marker, or neurofilament, a neuronal marker, were identified indicating that the MSCs differentiated into these cell types, although the question of whether MSCs may have fused with recipient brain cells was not addressed.

In summary, it is conclusively demonstrated that MSCs have the potential to differentiate to mesenchymal lineages both *in vivo* and *in vitro*. Animal studies have indicated that MSC may also have the potential to form non-mesenchymal cell types but definitive evidence of this ability has not yet been provided. This will require *in vitro* studies of clonal MSC populations incorporating phenotypic and functional assays, combined with *in vivo* investigations that also address the issue of cell fusion.

Isolation of MSCs from Site Other Than the Bone Marrow

In addition to their presence in bone marrow, cells with the characteristics of MSCs reside in other human tissues, including trabecular bone,[36] deciduous teeth,[37] skin and muscle[38] and synovial and periosteum.[39–41] However, MSC-like cells from adipose tissue and umbilical cord blood have received the most attention. Fibroblastic, plastic-adherent cell populations can be isolated from lipoaspirates (ADSC[42]) and placental blood (UBSC[43,44]) and are capable of being maintained *in vitro* for many population doublings. Clonal populations of both ADSCs and UBSCs show trilineage differentiation potential to adipocytes, osteocytes and chondrocytes,[42,44] although multipotential clones were reported to be rare in ADSC. ADSC can differentiate into adipocytes that functionally resembled white adipose tissue, as well as adipocytes from bone marrow-derived MSC.[45,46] Clonally-derived UBSC populations are reportedly capable of differentiation into hepatocyte-like and neural cells.[44]

STEM CELL REPAIR AND REGENERATION - Volume 3
© Imperial College Press
http://www.worldscibooks.com/lifesci/p535.html

Heterogeneous populations of ADSC and UBSC have been shown to differentiate into myogenic cells,[42,47,48] and ADSC may also be able to form hepatocyte-like[49] and neural cell lineages.[42,50] However, some methods used to induce neurogenesis in these studies have come under question, and clonal studies are required to ensure that no contaminating progenitor cells were present.

The relationship between bone marrow-derived MSCs, ADSCs and UBSCs has been investigated. Surface marker profiles were found to be comparable between all populations.[42,44,51] A microarray comparison found ADSCs and bone marrow MSCs to be similar, with only 25 genes from an array representing over 10,000 genes showing differential expression.[52] However, a second study showed numerous expression differences by pairwise comparisons of the three populations, but gene ontology analysis of the differentially expressed genes did not reveal gene expression trends characteristic of the tissue of origin.[51] MSCs can also be derived from adult synovial membrane and periosteum. Clonal populations of both synovial and periosteum-derived stem cells have the potential to differentiate into cartilage, bone, fat and muscle using both *in vitro* and *in vivo* models of differentiation.[39,40]

There have also been reports of cells isolated from normal whole blood which share a similar antigenic profile to MSCs and have the ability to differentiate into adipocytes and osteocytes.[53,54] An elegant study in rats by Wu *et al.*, captured circulating precursor cells that demonstrated adipogenic, osteogenic, chondrogenic and myogenic differentiation in culture, and when immortalised showed clonal differentiation to these lineages.[55] Furthermore, labelled clonal cells were shown to home to the bone marrow and also areas of injured cardiac tissue.[55] These studies indicate that a multipotential precursor cell, reminiscent of MSC, may circulate in the blood and have the ability to home to sites of injury or disease. This may also provide a link between the bone marrow and tissue-specific MSC-like populations, however this work remains controversial as other groups have been unable to replicate these studies therefore further investigation is required.

Multipotent Adult Progenitor Cells

Multipotent adult progenitor cells (MAPC) are a subpopulation of bone marrow cells that co-purify with MSCs isolated from human or rodent

STEM CELL REPAIR AND REGENERATION - Volume 3
© Imperial College Press
http://www.worldscibooks.com/lifesci/p535.html

bone marrow.[56,57] Colonies derived from single cells are able to differentiate *in vitro* into cells with phenotypic and functional characteristics of endothelial cells (visceral mesoderm), neurons, astrocytes and oligodendrocytes (neuroectoderm), and hepatocytes (endoderm), as well as cells of mesenchymal lineages.[56–60] Injection of murine MAPCs into an early mouse blastocyst generated chimeric offspring with MAPCs contributing to most somatic tissues.[57] It is thought that MAPCs represent a pluripotential stem cell that persists into adulthood and are capable of differentiating into cells of all three germ layers. However, it could not be ruled out that cell fusion may be responsible for the *in vivo* observations.[57] The relationship of MAPCs to hMSCs is also a matter for debate. The *in vitro* culture conditions of the two populations are very different; MAPCs are cultured at low densities and 2% FCS, whereas MSCs are cultured at higher densities and 10% FCS. It is possible that this contributes to the differences in potentiality observed, and that MAPCs are the result of dedifferentiation of MSCs due to culture conditions[61] (Elliman and Clements, unpublished data). Whether MAPCs truly exist as a pluripotential stem cell population *in vivo* is as yet unknown.

MSC Cell Surface Markers

MSCs are classically isolated by their adherence to tissue culture plastic which gives some degree of purification as non-adherent haematopoietic cells are removed.[16] However, a lack of knowledge regarding surface markers has precluded the use of more sensitive antibody-aided selection. Studies of surface markers of cultured human MSCs have shown that they are distinct from haematopoietic cells, as they do not express CD45, CD34 and CD14, markers of the haematopoietic lineage.[16,62] Numerous other surface molecules have been identified (Table 1), and indicate that hMSCs express markers of mesenchymal (SH2, SH3 and SH4, ACTA1), endothelial (VCAM-1) and epithelial (cytokeratins 18 and 19) lineages.[62] It must be noted however, that varied culture conditions used by different laboratories mean that contradictory results regarding MSC marker profiles are frequent.[61]

Unique markers are needed to definitively identify and purify non-expanded MSCs and to study their biology *in vivo*. The most promising candidate to date is the monoclonal antibody STRO-1. Its antigen (as yet unidentified) is expressed by a population of bone marrow stromal

STEM CELL REPAIR AND REGENERATION - Volume 3
© Imperial College Press
http://www.worldscibooks.com/lifesci/p535.html

Table 1. Surface marker expression profile of MSCs

Marker type	Marker name	
	Expressed	Not expressed
Specific antigens	SH2, SH3, SH4, Stro-1, ACTA1	CD133
Haematopoietic markers		CD4, CD14, CD34, CD45 (PTPRC), c-kit/SCFR (CD117)
Cytokines and growth factors	IL-1α, 6, 7, 8, 11, 12, 14, and 15 LIF, SCF, GM-CSF, G-CSF, M-CSF	
Cytokine and growth factor receptors	IL1R, IL3R, IL4R, IL6R, IL7R, LIFR, SCFR, G-CSFR, IFNγR, TNFR1, TNFR2, TGFβR1, TGFβR2, bFGFR, PDGFR, EGFR	IL-2R (CD25)
Adhesion molecules	Integrins α1 (CD49a), α2 (CD49b), α3 (CD49c), aα (CD49e), β1 (CD29), β3 (CD61), β4 (CD104)	α4 (CD49d), αL (CD11a), Cβ2 (CD18)
Extracellular matrix molecules and receptors	ICAM-1 (CD54), ICAM-2 (CD102), VCAM-1 (CD106), ALCAM-1 (CD166), LFA3 (CD58), L-selectin (CD62L), endoglin (CD105), hyaluronate (CD44) CK18, CK19	ICAM-3 (CD50), E-selectin (CD62E), P-selectin (CD62P), PECAM-1 (CD31), vWF, Cadherin 5
Others	CD9, CD13, Thy-1 (CD90), HLA-ABC (MHC I) (low)	HLA-DR (MHC II)

Data obtained from Pittenger *et al.*,[16] Minguel *et al.*,[73] Majumdar *et al.*[96] and Wagner *et al.*[51]

cells (termed CFU-Fs in these studies) that possesses the ability to proliferate extensively and differentiate *in vitro* into osteoblasts, adipocytes, chondrocytes and haematopoiesis-supportive stroma.[63,64] However, around 95% of this STRO-1$^+$ population constituted glycophorin A-positive

STEM CELL REPAIR AND REGENERATION - Volume 3
© Imperial College Press
http://www.worldscibooks.com/lifesci/p535.html

nucleated erythroid precursors, so a further glycophorin A^- selection was required to enrich for CFU-Fs.[63] Double selection for cells from whole human bone marrow expressing high levels of STRO-1 ($STRO1^{BRIGHT}$) and also VCAM1/CD106 resulted in 5000-fold enrichment of the CFU-F population.[65] A subset of $STRO1^{BRIGHT}VCAM^+$ cells exhibited extensive *in vitro* proliferation and clonal multipotentiality; as other clones exhibited low proliferation and differentiation, it was concluded that multipotential stem cells constituted a subset of the isolated population, which also contained more restricted progenitor cells.[65] CFU-Fs have also been enriched via selection of $CD45^-$, $CD14^-$, $CD73^+$/$CD49a^+$[66] or $CD45^{low}$, D7-FIB^+ (a fibroblast marker of unknown function,[67] populations from non-expanded bone marrow samples, but a pure CFU-F population was not obtained from either of these methods. Various approaches have been used to try and identify new definitive markers of MSCs including gene expression microarray profiling[68] and phage display technologies.[69] Combined with the cell surface marker studies, it is clear that progress is being made towards defining the MSC population, but there is as yet no marker that is unique to MSCs.

An exciting recent report by Anjos-Afonso and Bonnet[70] describes the isolation of a primitive population of murine mesenchymal progenitors based on the expression of stage-specific embryonic antigen-1 (SSEA-1). Single cell-derived clones not only gave rise to MSCs but were capable of differentiation to non-mesodermal cell types such as hepatocyte and neural lineages.[70] The authors propose that a hierarchical organisation of the mesenchymal compartment similar to HSCs with SSEA-1 progenitors being at the top. It will be interesting to see if a similar population of cells can be isolated from human bone marrow.

Self-Renewal of MSCs

A defining characteristic of stem cells is the ability to self-renew indefinitely. Although the term mesenchymal stem cell is widely used, no unequivocal evidence demonstrating *in vivo* self-renewal of MSC has yet been provided.[64,71] Although human MSC can be expanded for 15–25 passages *in vitro* (depending on culture conditions and donor age) without losing differentiation potential, this *in vitro* proliferative capacity is not indefinite.[62,72] Furthermore, it is possible that MSC *in vitro* represent

STEM CELL REPAIR AND REGENERATION - Volume 3
© Imperial College Press
http://www.worldscibooks.com/lifesci/p535.html

a heterogeneous population of multipotential stem cells and lineage-restricted progenitors; evidence from this comes from the studies of Gronthos and Pittenger where only a proportion of hMSC clones could differentiate into three lineages.[16,65] This could be a reflection of a stem cell hierarchy similar to HSC.[12,70,73] Furthermore, suboptimal culturing conditions *in vitro* may also cause loss of potentiality of a proportion of multipotential cells, leading to a heterogeneous population. Definitive proof of MSC self-renewal and multipotentiality will come from the isolation of a highly purified cell population followed by demonstration that, at the single cell level, unmanipulated MSCs could give rise *in vivo* to diverse progeny and re-populate host animals after serial transplantation.[74] Currently the only adult stem cell population for which these rigorous criteria have been demonstrated is the HSC, and the lack of a MSC-specific marker precludes such work in this population. However, various observations from cultured or freshly isolated MSCs point towards a stem cell function.

Clonal populations of human MSCs are capable of giving rise robustly to multiple lineages *in vitro*, arguing strongly for the presence of a multipotential stem cell.[16,65] In addition, studies of freshly isolated, non-expanded MSCs revealed that they expressed Ki-67 antigen, a marker of non-cycling cells, and were mostly in G0/G1 phase of the cell cycle, entering the cell cycle after exposure to serum in culture.[8,62,65] This suggests that MSC *in vivo* are a resting cell population, a characteristic of other stem cell populations.[75] Freshly isolated MSC were also found to exhibit telomerase activity[65] which persisted in culture for at least 12 passages;[16] this is again a characteristic of stem cells with self-renewal capacity.

Signalling Pathways Involved in MSC Proliferation and Differentiation

The molecular regulation of stem cell proliferation and differentiation is very complex involving the interaction and cross-talk between different signalling pathways via the release of specific growth factors.[76] In tissues, the equilibrium between stem cell proliferation and differentiation is tightly controlled to ensure normal tissue function. Uncontrolled proliferation of stem cells can give rise to cancer,[77] whereas depletion of the stem cell pool by aberrant differentiation

STEM CELL REPAIR AND REGENERATION - Volume 3
© Imperial College Press
http://www.worldscibooks.com/lifesci/p535.html

leads to tissue degeneration.[78] Understanding the signalling pathways involved in regulating proliferation and differentiation of stem cells is fundamental to the exploitation of these cells for therapeutic applications.

Little is known about the signalling pathways that control MSC behaviour. Differentiation to specific cell lineages *in vitro* is induced by the addition of defined combinations of growth factors and supplements.[16] The first growth factor found to enhance the proliferation of human MSCs was basic fibroblast growth factor (bFGF). The addition of bFGF to MSC cultures increased both the growth rate and life span of MSC without altering their differentiation potential.[17] More recently it was reported that the addition of bFGF was optimal for large scale production of MSCs, with cells retaining their differentiation and immunosuppressive capacities.[79]

Another pathway reported to control the proliferation of human MSCs is Wnt signalling. Wnts are a family of factors that regulate cell growth and cell fate in a variety of cell types.[80] Signalling is regulated via the binding of ligands to the Frizzled family of transmembrane receptors. This leads to the stabilisation of β-catenin which translocates to the nucleus where it binds the TCF/LEF family of transcription factors to regulate the expression of Wnt target genes. Activation of Wnt signaling in MSCs by lithium chloride treatment or Wnt3a conditioned media is complex. Low levels of Wnt signalling are mitogenic promoting MSC proliferation, however, high levels have the opposite affect and result in growth arrest.[81] This growth arrest was associated with an increased expression of osteogenic markers and a decreased potential to form adipocytes. Human MSCs also secrete Dkk-1, an inhibitor of the Wnt pathway. Addition of Dkk-1 peptides promoted the recovery of MSCs from the bone marrow and increased their profileration rate,[82] although it blocked the osteogenic potential of MSCs.

Notch signalling is another important pathway involved in the coordination of development and is present in all animal species studied to date.[83,84] The pathway was first identified in Drosophila where mutations in Notch signalling affected neurogenesis. In mammals, Notch signalling is mediated by cell-to-cell contact which brings together the membrane-bound Notch ligands (Delta-like-1, -3 and -4; Jagged-1 and -2) with the Notch receptors (Notch 1-4). Binding of the ligand to the receptor results in proteolytic cleavage of the Notch receptor, releasing the Notch intracellular domain (NICD) which then translocates to the

STEM CELL REPAIR AND REGENERATION - Volume 3
© Imperial College Press
http://www.worldscibooks.com/lifesci/p535.html

nucleus where it interacts with the transcription factor CSL, switching its transcriptional activity from a repressor to an activator. The proteolytic cleavage of Notch is a multistep process involving the γ-secretase complexes presenilin-1 and -2.

Notch signalling is implicated in a wide range of mammalian developmental processes including neurogenesis, vascular formation and limb bud development[83] and has recently been shown to promote neural lineage entry of embryonic stem cells.[85] There is growing evidence that Notch signalling also plays an important role in mesoderm specification. Homozygous knock-out mice for the Notch ligand Jagged-2 have defects in limb and craniofacial development. Notch signalling is also important during human development as mutations in Jagged-1 give rise to the skeletal defects in Alagilles syndrome[86,87] while mutations in Delta-like-3 are associated with dwarfism in spondylocostal dysostosis.[88] In murine chondrogenic and osteoblast cell lines, the over-expression of Delta-1 or NICD inhibits both chondrogenesis[89] and osteogenic differentiation,[90] whereas the role of Notch signalling in adipocyte formation is more complex. Translocation of NCID in the murine 3T3L1 pre-adipocyte cell line is required for adipogenesis,[91] whereas either over-expression or inhibition of HES-1 (downstream target of Notch signalling) blocks differentiation[91,92] implying a dual role for Notch during adipogenesis. It is therefore apparent that Notch signalling is important during mesoderm development but its role is complex and requires further studies to precisely elucidate its role of action.

Human MSCs provide an ideal model system to investigate the role of Notch signalling during mesoderm development. Unlike murine cell lines previously studied, MSCs are primary cells and thus do not harbour mutations that may alter normal Notch signalling responses. In addition, the multipotent nature of MSCs permits the study of differentiation towards three mesenchymal lineages using a single cell type, overcoming the problem of comparing results between different lineage-specific cell line models. Gene expression microarray profiling studies in our laboratory indicate that the expression of genes involved in Notch signalling change upon MSC differentiation (Vujovic, Westwood and Clements, unpublished observations). The role of Notch signalling in regulating proliferation and differentiation of MSCs was investigated by inhibiting γ-secretases involved in processing of the Notch receptor after activation of the pathway.[93] The addition of DAPT (N-[N-(3,5-difluorophenacetyl)-L-alanyl]-(S)-phenylglycine t-butyl ester) to proliferating MSCs led to a

STEM CELL REPAIR AND REGENERATION - Volume 3
© Imperial College Press
http://www.worldscibooks.com/lifesci/p535.html

reduction in their proliferative capacity and altered their differentiation potential. During chondrogenesis, DAPT reduced extracellular matrix production, but was inductive for adipogenic differentiation. Although DAPT is a specific γ-secretase inhibitor, γ-secretases have been shown to have Notch-independent functions.[94] However, the reduced chondrogenic differentiation observed was associated with a decrease in the levels of HES1 and HEY1 indicative of Notch-dependent effects. Further characterization of the role of Notch signalling in MSC proliferation and differentiation is underway using viral vectors[95] to modulate the expression of key components of the Notch signalling pathway.

Conclusion

Our understanding of MSC biology is gradually increasing although many challenges lie ahead before we can realise the full therapeutic potential of these cells. This review has highlighted some of the key areas for future investigation which include the identification of specific markers to enable the isolation of pure populations of MSCs, as well as a better understanding of the regulatory pathways that control MSC behaviour. Advances in these areas will increase our basic knowledge of mesenchymal development as well as facilitate the development of MSC-based therapies.

References

1. Caplan AI, Bruder SP. Mesenchymal stem cells: building blocks for molecular medicine in the 21st century. *Trends Mol Med* 2001;7(6):259–264.
2. Oreffo RO, Cooper C, *et al*. Mesenchymal stem cells: lineage, plasticity, and skeletal therapeutic potential. *Stem Cell Rev* 2005;1(2):169–178.
3. Urist M, Mclean F. Osteogenetic potency and new-bone formation by induction in transplants to the anterior chamber of the eye. *J Bone Joint Surg Am* 1952;34-A(2): 443–476.
4. Tavassoli M, Crosby WH. Transplantation of marrow to extramedullary sites. *Science* 1968;161(836):54–56.
5. Friedenstein AJ, Piatetzky-Shapiro II, *et al*. Osteogenesis in transplants of bone marrow cells. *J Embryol Exp Morphol* 1966;16(3):381–390.
6. Friedenstein AJ, Chailakhjan RK, *et al*. The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. *Cell Tissue Kinet* 1970;3(4):393–403.

© Imperial College Press

7. Friedenstein AJ. Precursor cells of mechanocytes. *Int Rev Cytol* 1976;47:327–359.
8. Castro-Malaspina H, Gay RE, *et al*. Characterization of human bone marrow fibroblast colony-forming cells (CFU-F) and their progeny. *Blood* 1980;56(2):289–301.
9. Ashton BA, Allen TD, *et al*. Formation of bone and cartilage by marrow stromal cells in diffusion chambers *in vivo*. *Clin Orthop* 1980;151:294–307.
10. Friedenstein AJ. Stromal mechanisms of bone marrow: Cloning *in vitro* and retransplantation *in vivo*. *Haematol Blood Transfus* 1980;25:19–29.
11. Owen M. Histogenesis of bone cells. *Calcif Tissue Res* 1978;25(3):205–207.
12. Owen M. Lineage of osteogenic cells and their relationship to the stromal system. In: Peck WA (ed.), *Bone and Mineral Research Volume 3*. (Elsevier, Amsterdam, 1985), pp. 1–25.
13. Owen M. Marrow stromal stem cells. *J Cell Sci Suppl* 1988;10:63–76.
14. Caplan AI. Mesenchymal stem cells. *J Orthop Res* 1991;9(5):641–650.
15. Dennis JE, Merriam A, *et al*. A quadripotential mesenchymal progenitor cell isolated from the marrow of an adult mouse. *J Bone Miner Res* 1999;14(5):700–709.
16. Pittenger MF, Mackay AM, *et al*. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999;284(5411):143–147.
17. Tsutsumi S, Shimazu A, *et al*. Retention of multilineage differentiation potential of mesenchymal cells during proliferation in response to FGF. *Biochem Biophys Res Commun* 2001;288(2):413–419.
18. Young RG, Butler DL, *et al*. Use of mesenchymal stem cells in a collagen matrix for Achilles tendon repair. *J Orthop Res* 1998;16(4):406–413.
19. Zimmet JM, Hare JM. Emerging role for bone marrow derived mesenchymal stem cells in myocardial regenerative therapy. *Basic Res Cardiol* 2005;100(6):471–481.
20. Makino S, Fukuda K, *et al*. Cardiomyocytes can be generated from marrow stromal cells *in vitro*. *J Clin Invest* 1999;103(5):697–705.
21. Xu W, Zhang X, *et al*. Mesenchymal stem cells from adult human bone marrow differentiate into a cardiomyocyte phenotype *in vitro*. *Exp Biol Med* 2004;229(7): 623–631.
22. Wang PP, Wang JH, *et al*. Expression of hepatocyte-like phenotypes in bone marrow stromal cells after HGF induction. *Biochem Biophys Res Commun* 2004; 320(3):712–716.
23. Ong SY, Dai H, *et al*. Hepatic differentiation potential of commercially available human mesenchymal stem cells. *Tissue Eng* 2006:12(12):3477–3487.
24. Woodbury D, Schwarz EJ, *et al*. Adult rat and human bone marrow stromal cells differentiate into neurons. *J Neurosci Res* 2000;61(4):364–370.
25. Hung SC, Cheng H, *et al*. *In vitro* differentiation of size-sieved stem cells into electrically active neural cells. *Stem Cells* 2002;20(6):522–529.
26. Zurita M, Vaquero J, *et al*. Schwann cells induce neuronal differentiation of bone marrow stromal cells. *Neuroreport* 2005;16(5):505–508.
27. Lu P, Blesch A, *et al*. Induction of bone marrow stromal cells to neurons: differentiation, transdifferentiation, or artifact? *J Neurosci Res* 2004;77(2):174–191.
28. Neuhuber B, Gallo G, *et al*. Re-evaluation of *in vitro* differentiation protocols for bone marrow stromal cells: disruption of actin cytoskeleton induces rapid morphological changes and mimics neuronal phenotype. *J Neurosci Res* 2004; 77(2):192–204.

STEM CELL REPAIR AND REGENERATION - Volume 3
© Imperial College Press
http://www.worldscibooks.com/lifesci/p535.html

29. Sanchez-Ramos J, Song S, *et al*. Adult bone marrow stromal cells differentiate into neural cells *in vitro*. *Exp Neurol* 2000;164(2):247–256.
30. Hermann A, Gastl R, *et al*. Efficient generation of neural stem cell-like cells from adult human bone marrow stromal cells. *J Cell Sci* 2004;117(Pt 19):4411–4422.
31. Pereira RF, Halford KW, *et al*. Cultured adherent cells from marrow can serve as long-lasting precursor cells for bone, cartilage, and lung in irradiated mice. *Proc Natl Acad Sci USA* 1995;92(11):4857–4861.
32. Pereira RF, O'Hara MD, *et al*. Marrow stromal cells as a source of progenitor cells for nonhematopoietic tissues in transgenic mice with a phenotype of osteogenesis imperfecta. *Proc Natl Acad Sci USA* 1998;95(3):1142–1147.
33. Anjos-Afonso F, Siapati EK, *et al*. *In vivo* contribution of murine mesenchymal stem cells into multiple cell-types under minimal damage conditions. *J Cell Sci* 2004; 117(Pt 23):5655–5664.
34. Liechty KW, MacKenzie TC, *et al*. Human mesenchymal stem cells engraft and demonstrate site-specific differentiation after *in utero* transplantation in sheep. *Nat Med* 2000;6(11):1282–1286.
35. Kopen GC, Prockop DJ, *et al*. Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. *Proc Natl Acad Sci USA* 1999;96(19):10711–10716.
36. Noth U, Osyczka AM, *et al*. Multilineage mesenchymal differentiation potential of human trabecular bone-derived cells. *J Orthop Res* 2002;20(5):1060–1069.
37. Miura M, Gronthos S, *et al*. SHED: stem cells from human exfoliated deciduous teeth. *Proc Natl Acad Sci USA* 2003;100(10):5807–5812.
38. Young HE, Steele TA, *et al*. Human reserve pluripotent mesenchymal stem cells are present in the connective tissues of skeletal muscle and dermis derived from fetal, adult, and geriatric donors. *Anat Rec* 2001;264(1):51–62.
39. DeBari C, Dell'accio F, *et al*. Multipotent mesenchymal stem cells from adult human synovial membrane. *Arthritis Rheum* 2001;44(8):1928–1942.
40. DeBari C, Dell'accio F, *et al*. Mesenchymal multipotency of adult human periosteal cells demonstrated by single-cell lineage analysis. *Arthritis Rheum* 2006;54(4):1209–1221.
41. DeBari C, Dell'accio F, *et al*. Human periosteum-derived cells maintain phenotypic stability and chondrogenic potential throughout expansion regardless of donor age. *Arthritis Rheum* 2001;44(1):85–95.
42. Zuk PA, Zhu M, *et al*. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell* 2002;13(12):4279–4295.
43. Erices A, Conget P, *et al*. Mesenchymal progenitor cells in human umbilical cord blood. *Br J Haematol* 2000;109(1):235–242.
44. Lee OK, Kuo TK, *et al*. Isolation of multipotent mesenchymal stem cells from umbilical cord blood. *Blood* 2004;103(5):1669–1675.
45. Rodriguez AM, Elabd C, *et al*. Adipocyte differentiation of multipotent cells established from human adipose tissue. *Biochem Biophys Res Commun* 2004;315(2): 255–263.
46. Dicker A, Le BK, *et al*. Functional studies of mesenchymal stem cells derived from adult human adipose tissue. *Exp Cell Res* 2005;308(2):283–290.

© Imperial College Press
http://www.worldscibooks.com/lifesci/p535.html

47. Zuk PA, Zhu M, *et al*. Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng* 2001;7(2):211–228.
48. Gang EJ, Jeong JA, *et al*. Skeletal myogenic differentiation of mesenchymal stem cells isolated from human umbilical cord blood. *Stem Cells* 2004;22(4): 617–624.
49. Seo MJ, Suh SY, *et al*. Differentiation of human adipose stromal cells into hepatic lineage *in vitro* and *in vivo*. *Biochem Biophys Res Commun* 2005;328(1):258–264.
50. Safford KM, Hicok KC, *et al*. Neurogenic differentiation of murine and human adipose-derived stromal cells. *Biochem Biophys Res Commun* 2002;294(2):371–379.
51. Wagner W, Wein F, *et al*. Comparative characteristics of mesenchymal stem cells from human bone marrow, adipose tissue, and umbilical cord blood. *Exp Hematol* 2005;33(11):1402–1416.
52. Lee RH, Kim B, *et al*. Characterization and expression analysis of mesenchymal stem cells from human bone marrow and adipose tissue. *Cell Physiol Biochem* 2004; 14(4–6):311–324.
53. Zvaifler NJ, Marinova-Mutafchieva L, *et al*. Mesenchymal precursor cells in the blood of normal individuals. *Arthritis Res* 2000;2(6):477–488.
54. Kuznetsov SA, Mankani MH, *et al*. Circulating skeletal stem cells. *J Cell Biol* 2001;153(5):1133–1140.
55. Wu GD, Nolta JA, *et al*. Migration of mesenchymal stem cells to heart allografts during chronic rejection. *Transplantation* 2003;75(5):679–685.
56. Reyes M, Lund T, *et al*. Purification and *ex vivo* expansion of postnatal human marrow mesodermal progenitor cells. *Blood* 2001;98(9):2615–2625.
57. Jiang Y, Jahagirdar BN, *et al*. Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* 2002;418(6893):41–49.
58. Reyes M, Dudek A, *et al*. Origin of endothelial progenitors in human postnatal bone marrow. *J Clin Invest* 2002;109(3):337–346.
59. Schwartz RE, Reyes M, *et al*. Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells. *J Clin Invest* 2002;109(10): 1291–1302.
60. Jiang Y, Vaessen B, *et al*. Multipotent progenitor cells can be isolated from postnatal murine bone marrow, muscle, and brain. *Exp Hematol* 2002;30(8):896–904.
61. Bonnet D. Biology of human bone marrow stem cells. *Clin Exp Med* 2003;3(3): 140–149.
62. Conget PA, Minguell JJ. Phenotypical and functional properties of human bone marrow mesenchymal progenitor cells. *J Cell Physiol* 1999;181(1):67–73.
63. Simmons PJ, Torok-Storb B. Identification of stromal cell precursors in human bone marrow by a novel monoclonal antibody, STRO-1. *Blood* 1991;78(1):55–62.
64. Dennis JE, Carbillet JP, *et al*. The STRO-1+ marrow cell population is multipotential. *Cells Tissues Organs* 2002;170(2-3):73–82.
65. Gronthos S, Zannettino AC, *et al*. Molecular and cellular characterisation of highly purified stromal stem cells derived from human bone marrow. *J Cell Sci* 2003;116 (Pt 9):1827–1835.
66. Boiret N, Rapatel C, *et al*. Characterization of non-expanded mesenchymal progenitor cells from normal adult human bone marrow. *Exp Hematol* 2005;33(2):219–225.

STEM CELL REPAIR AND REGENERATION - Volume 3
© Imperial College Press
http://www.worldscibooks.com/lifesci/p535.html

67. Jones EA, Kinsey SE, *et al*. Isolation and characterization of bone marrow multipotential mesenchymal progenitor cells. *Arthritis Rheum* 2002;46(12):3349–3360.
68. Ishii M, Koike C, *et al*. Molecular markers distinguish bone marrow mesenchymal stem cells from fibroblasts. *Biochem Biophys Res Commun* 2005;332(1):297–303.
69. Letchford J, Cardwell AM, *et al*. Isolation of C15: a novel antibody generated by phage display against mesenchymal stem cell-enriched fractions of adult human marrow. *J Immunol Methods* 2006;308(1–2):124–137.
70. Anjos-Afonso F, Bonnet D. Non-hematopoietic/endothelial SSEA-1pos cells defines the most primitive progenitors in the adult murine bone marrow mesenchymal compartment. *Blood* 2006;109(3):1298–1306.
71. Baksh D, Song L, *et al*. Adult mesenchymal stem cells: characterization, differentiation, and application in cell and gene therapy. *J Cell Mol Med* 2004;8(3):301–316.
72. Bruder SP, Jaiswal N, *et al*. Growth kinetics, self-renewal, and the osteogenic potential of purified human mesenchymal stem cells during extensive subcultivation and following cryopreservation. *J Cell Biochem* 1997;64(2):278–294.
73. Minguell JJ, Erices A, *et al*. Mesenchymal stem cells. *Exp Biol Med* 2001;226(6):507–520.
74. Joseph NM, Morrison SJ. Toward an understanding of the physiological function of Mammalian stem cells. *Dev Cell* 2005;9(2):173–183.
75. Taylor G, Lehrer MS, *et al*. Involvement of follicular stem cells in forming not only the follicle but also the epidermis. *Cell* 2000;102(4):451–461.
76. Morrison SJ, Shah NM, *et al*. Regulatory mechanisms in stem cell biology. *Cell* 1997;88(3):287–298.
77. Dalerba P, Cho RW, Clarke MF. Cancer stem cells: models and concepts. *Annu Rev Med* 2006;58:267–284.
78. Wagers AJ, Weissman IL. Plasticity of adult stem cells. *Cell* 2004;116(5):639–648.
79. Sotiropoulou PA, Perez SA, *et al*. Characterization of the optimal culture conditions for clinical scale production of human mesenchymal stem cells. *Stem Cells* 2006;24(2):462–471.
80. Cadigan KM, Nusse R. Wnt signaling: a common theme in animal development. *Genes Dev* 1997;11(24):3286–3305.
81. De BJ, Wang HJ, Van BC. Effects of Wnt signaling on proliferation and differentiation of human mesenchymal stem cells. *Tissue Eng* 2004;10(3–4):393–401.
82. Gregory CA, Perry AS, *et al*. Dkk-1-derived synthetic peptides and lithium chloride for the control and recovery of adult stem cells from bone marrow. *J Biol Chem* 2005;280(3):2309–2323.
83. Lai EC. Notch signaling: control of cell communication and cell fate. *Development* 2004;131(5):965–973.
84. Bray SJ. Notch signalling: a simple pathway becomes complex. *Nat Rev Mol Cell Biol* 2006;7(9):678–689.
85. Lowell S, Benchoua A, *et al*. Notch promotes neural lineage entry by pluripotent embryonic stem cells. *PLoS Biol* 2006;4(5):e121.
86. Li L, Krantz ID, *et al*. Alagille syndrome is caused by mutations in human Jagged1, which encodes a ligand for Notch1. *Nat Genet* 1997;16(3):243–251.
87. Oda T, Elkahloun AG, *et al*. Mutations in the human Jagged1 gene are responsible for Alagille syndrome. *Nat Genet* 1997;16(3):235–242.

STEM CELL REPAIR AND REGENERATION - Volume 3
© Imperial College Press
http://www.worldscibooks.com/lifesci/p535.html

88. Bulman MP, Kusumi K, *et al*. Mutations in the human delta homologue, DLL3, cause axial skeletal defects in spondylocostal dysostosis. *Nat Genet* 2000;24(4):438–441.
89. Watanabe N, Tezuka Y, *et al*. Suppression of differentiation and proliferation of early chondrogenic cells by Notch. *J Bone Miner Metab* 2003;21(6):344–352.
90. Sciaudone M, Gazzerro E, *et al*. Notch 1 impairs osteoblastic cell differentiation. *Endocrinology* 2003;144(12):5631–5639.
91. Garces C, Ruiz-Hidalgo MJ, *et al*. Notch-1 controls the expression of fatty acid-activated transcription factors and is required for adipogenesis. *J Biol Chem* 1997;272(47):29729–29734.
92. Ross DA, Rao PK, *et al*. Dual roles for the Notch target gene Hes-1 in the differentiation of 3T3-L1 preadipocytes. *Mol Cell Biol* 2004;24(8):3505–3513.
93. Vujovic S, Henderson SR, *et al*. Inhibition of gamma-secretases alters both proliferation and differentiation of mesenchymal stem cells. *Cell Prolif* 2007;40(2):185–195.
94. Kopan R, Ilagan MX. Gamma-secretase: proteasome of the membrane? *Nat Rev Mol Cell Biol* 2004;5(6):499–504.
95. Clements MO, Godfrey A, *et al*. Lentiviral manipulation of gene expression in human adult and embryonic stem cells. *Tissue Eng* 2006;12(7):1741–1751.
96. Majumdar MK, Keane-Moore M, Buyaner D, Hardy WB, Moorman MA, McIntosh KR, Mosca JD. Characterization and functionality of cell surface molecules on human mesenchymal stem cells. *J Biomed Sci* 2003;10(2):228–241.

STEM CELL REPAIR AND REGENERATION - Volume 3
© Imperial College Press
http://www.worldscibooks.com/lifesci/p535.html