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From fibroblasts to medicinal signalling cells: a paradigm shift in translational stem cell research

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Mesenchymal stem cells (MSCs) are fibroblast-like cells that give rise to mesenchymal derivatives including bone, fat and cartilage cells. In addition to the bone marrow, they can be readily obtained from adult vascularised peripheral tissues.

BIOGRAPHY

DR JONATHAN SHEARD is a postdoctoral researcher for Sheard BioTech Ltd, working to provide expertise in Biomedical Research, Stem Cell Biology and Advanced Imaging Techniques. He is currently based at the University of Reading working alongside the Widera Lab, investigating the use and development of a novel scaffold for biomedical research applications. He completed his PhD in Biomedical Sciences at Aston University, focusing on the identification and safe use of mesenchymal stromal cells towards clinical application, using high-content screening and live cell imaging systems.

MSCs and MSC-like cells can be isolated from the amniotic fluid and neonatal tissues such as umbilical cord and placenta. In general, MSCs are well tolerated, non-tumourigenic and the transplantation of these cells appears to be beneficial in a variety of diseases and conditions. Their high availability and relatively low-risk profile made MSCs a promising stem cell source for clinical exploitation.[1] Several *in vitro* studies claimed that MSCs can cross the germ layer boundary and give rise to neuronal (ectodermal) and pancreatic islet-like cells (endoderm).

However, most pre-clinical transplantation studies demonstrated negligible numbers of surviving MSCs in the host tissue and lack of differentiation into non-mesodermal cell types. Nowadays, it is widely accepted that MSCs lack the ability to generate progeny beyond bone, fat, and cartilage cells and that their level of engraftment after transplantation is extremely low. Nevertheless, MSCs have been unequivocally shown to mediate beneficial effects in many animal models of disease and in clinical conditions. The current understanding of their mode of action includes: a reduction of inflammation, immune-modulation, and an increase of the endogenous regeneration of the host tissue. Moreover, all these 'bystander effects' are mediated by paracrine factors secreted by the MSCs. Briefly, the paracrine factors can be soluble or embedded within nano-scale extracellular vesicles. In the following, we discuss the journey from bone marrow-derived fibroblast-like cells to the modern understanding of MSCs as medicinal signalling cells and, critically, discuss the clinical and commercial impact of the factors secreted by these cells.

Fibroblast colony-forming units

The suggestion of a stem cell-like population as the origin of non-haematopoietic bone marrow cells, which contribute to wound repair and have a fibroblastic-like morphology, was made almost 150 years ago.[2,3] The subsequent work conducted from the late 1960s through the 1970s, credited the discovery of MSCs. Dr JF Cronheim made the critical observation that the bone marrow, in postnatal life, is a reservoir of stem cells for mesenchymal tissues. Briefly, he isolated adherent, clonogenic, fibroblast-like cells from the rodent bone marrow, termed them 'fibroblast colony-forming units' and demonstrated that these cells possess high replicative potential *in vitro* (CFU-F).[4-7] Later, CFU-F were shown to be able to undergo mineralisation *in vitro*[8,9] and to have osteogenic differentiation potential *in vivo*.[10]

Mesenchymal stem cells

In 1991, based on the embryonic perspective of multipotent progenitor cells located within the mesodermal layer, Caplan postulated that a population of mesenchymal cells able to give rise to a spectrum of mesenchymal cells is present in adult bone marrow.[11] Indeed, in 1999, Pittenger verified this hypothesis by showing that cells isolated from human adult bone marrow aspirates display a stable phenotype in an *in vitro* monolayer culture and can be induced to differentiate into the osteogenic, adipogenic, and chondrogenic lineages (***Figure 1***).[12] This multi-lineage differentiation potential led to the assumption that this stromal cell population has stem cell properties. Consequently, MSCs have been termed mesenchymal stem cells.

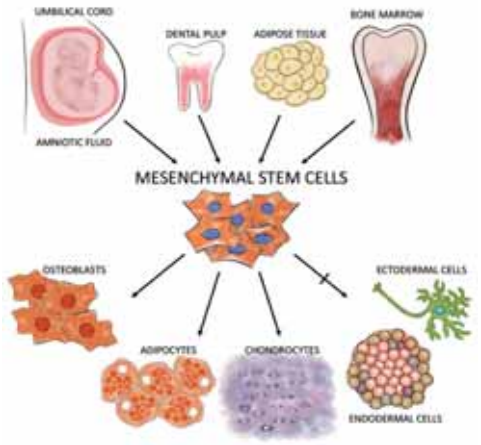
Mesenchymal stromal cells

In 2006, to standardise the nomenclature and to allow for a comparison of scientific studies among different laboratories, the International Society for Cellular Therapy (ISCT) introduced a set of minimal criteria for MSCs. According to this definition, MSCs must be plastic adherent, able to differentiate into adipogenic, chondrogenic and osteogenic cells, and should express CD105, CD73 and CD90 cell-surface markers. Moreover, MSCs are defined by the lack of the hematopoietic markers CD45, CD34, CD14, CD11b, CD79α, CD19, and HLA-DR.[13] More importantly, in this position paper, the ISCT recommended the use of term multipotent mesenchymal stromal cells (MSC) instead of mesenchymal stem cells. Notably, this change in nomenclature has not been justified by arguments addressing the stem cell characteristics of MSCs.

MSCs: more than multipotent?

In the following years, a multitude of studies suggested that MSCs might be able to cross the germ layer boundaries and differentiate into ectodermal and endodermal cells. In particular, neuronal differentiation has been claimed by several independent laboratories.[14-18] However, most of the studies relied heavily on morphology and marker expression and did not provide functional data in direct comparison to neurons. Notably, MSCs display a neuron-like morphology after exposure to cellular stress and express neuronal markers even after differentiation into osteogenic and adipogenic fate.[19] The lack of neuronal differentiation has also been supported by several studies showing no functional integration and differentiation of MSCs in the brain tissue. Similarly, it has been postulated that MSCs could undergo endodermal differentiation into pancreatic islet-like cells.[15,20] However, the authors did not provide functional proof of differentiation. Despite several attempts to prove cross-lineage differentiation, there is little evidence for the claim that MSCs can give rise to more than mesenchymal cells without direct programming using forced expression of ectodermal and endodermal factors. This relatively low differentiation potential and the negligible level of engraftment contrast with the promising pre-clinical and clinical results after MSC transplantation. Briefly, MSCs have been shown to alleviate symptoms of a broad spectrum of diseases and symptoms including liver cirrhosis (affected germ layer: endoderm),[21] severe ischaemic heart failure (affected germ layer: mesoderm),[22] and progressive Multiple Sclerosis (affected germ layer: ectoderm).[23] This discrepancy led to the conclusion that the therapeutic effects of MSC-administration are a result of paracrine bystander effects rather than a consequence of an engraftment and differentiation towards lost cell types.[24-29]

FIGURE 1



LEFT: Isolation and differentiation potential of MSCs

The illustration shows examples of known niches of MSCs – the amniotic fluid, umbilical cord, dental pulp, adipose tissue, and bone marrow. The standard ISCT minimal criteria of tri-lineage differentiation of these isolated MSCs along osteoblastic, adipogenic and chondrogenic lineages is also shown. The controversial claim of MSCs' potential to cross the germ layer boundaries and differentiate into ectodermal (neuron-like cells) and endodermal cells (pancreatic-islet like cells) is shown with a crossed arrow

Medicinal signalling cells

The prominent bystander effects mediated by MSCs led to a newer definition describing these cells as 'Medicinal signalling cells'.[30] According to this definition, MSCs function as surveyors of their microenvironments. During local injury, they are released from their niche, become activated, and establish a regenerative microenvironment by secreting bioactive molecules and regulating the local immune response. If transplanted into a side of injury or degeneration, MSCs sense the injury signals and release paracrine factors contributing thereby to the endogenous regeneration process in the host. These trophic and immunomodulatory activities suggest that MSCs may indeed serve as site-regulated `drugstores` *in vivo*.[31,32]

Secretome of MSCs as an emerging alternative to MSC transplantation

The regenerative competency of MSCs is most likely mediated by paracrine factors within the MSC-secretome, as described above. This is evidenced by a similar tissue repair and regeneration potential of MSC-derived conditioned medium (CM) compared with MSC transplantation.[33-35] In the following, we briefly discuss the active compounds within the CM focusing on soluble proteins and the cargo of extracellular vesicles (EVs) released by MSCs.

Soluble factors

Secretion of soluble factors is at least partly responsible for the two key therapeutic modes underlying tissue repair mechanisms mediated by MSCs, namely: immunosuppressive / modulatory and tissue regeneration effects. Notably, MSCs do not confer an immunosuppressive effect in a constitutive manner, but rather in response to the local tissue microenvironment. More specifically, MSCs are able to sense inflammation and tissue damage via tumour necrosis factor receptors (TNFRs)[36] and toll-like

BIOGRAPHY

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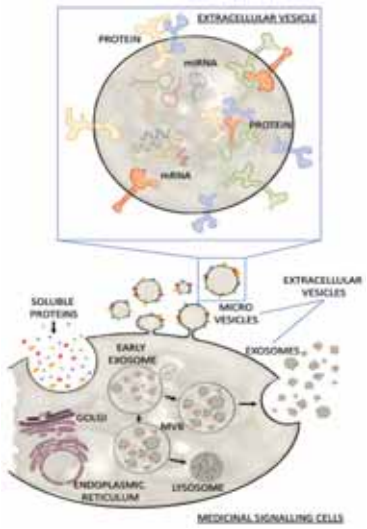
RIGHT: Isolation and differentiation potential of MSCs

The illustration shows examples of known niches of MSCs – the amniotic fluid, umbilical cord, dental pulp, adipose tissue, and bone marrow. The standard ISCT minimal criteria of tri-lineage differentiation of these isolated MSCs along osteoblastic, adipogenic and chondrogenic lineages is also shown. The controversial claim of MSCs' potential to cross the germ layer boundaries and differentiate into ectodermal (neuron-like cells) and endodermal cells (pancreatic-islet like cells) is shown with a crossed arrow

FIGURE 2



receptors (TLRs),[37,38] resulting in the release of an array of soluble factors including human leukocyte antigen G (HLA-G), IL-6, IL-10, indoleamine 2,3-dioxygenase (IDO), nitric oxide (NO) and TGF-β. These soluble factors elicit immunosuppressive and immunomodulatory actions by regulating the proliferation and function of a variety of immune cells.[39,40]

A broad spectrum of cytokines, chemokines and growth factors (EGF, TGF-β, IGF-1, HGF, VEGF) secreted by MSCs are also known to stimulate molecular and cellular processes involved in regeneration, including proliferation of host cells, angiogenesis, chemo-attraction and tissue remodelling. Interestingly, *in vitro* exposure of MSCs to pro-inflammatory factors (licencing) has been shown to increase the regenerative potential of MSCs.[41] Moreover, it has been demonstrated that relatively high levels of anti-apoptotic factors are present in bone marrow MSC-CM and that these soluble factors protect pancreatic cells from apoptosis in a diabetic animal model.[42] Intriguingly, CM of adult periodontal ligament MSCs from young donors was shown to increase proliferation of aged MSCs *in vitro* and *in vivo*. This effect has been largely attributed to secreted soluble growth and differentiation factors including IGF-1, PDGF, IL-1, TGF-β, BMP-2 and BMP-4.

Neither single soluble factors alone nor combinations thereof can fully recapitulate the therapeutic effects of MSCs. This can be explained by the fact that MSCs act as 'cytokine biofactories' that adapt their products in response to external cues. Moreover, over the past decade, the paracrine effects of MSCs have also been shown to be at least partly mediated by EVs.

Extracellular vesicles

The term 'extracellular vesicle' is an umbrella term for a heterogeneous population of vesicles secreted by all animal and human cell types. At least two types of these vesicles, exosomes[44] and microvesicles,[45] have been shown to contribute to the regenerative potential of MSC-secretome. Briefly, exosomes are nanoscale vesicles of endosomal origin with a size ranging from 30 to 100nm, while microvesicles bud from the plasma membrane and are slightly larger (100–100nm) (***Figure 2***).[46] With the existing technologies, microvesicles and exosomes can hardly be distinguished. Consequently, the International Society of Extracellular Vesicles (ISEV) suggested using the term EV for both types of vesicles.[46] Currently, differential centrifugation represents the most widely used method for EV isolation.[47,48] This method includes centrifugation at low speed to remove cell debris and high-speed ultracentrifugation to purify EVs. However, it has been reported that ultracentrifugation can negatively affect the integrity of exosomes.[49] Despite the lack of standard isolation methods, EVs have been shown to harbour a regenerative potential that is similar to MSC-mediated effects. Direct side-by-side comparisons in models of acute kidney failure, ischemic stroke, and lung injury revealed that MSCs and MSC-derived EVs exert comparable regenerative effects.[50-53] Despite these exciting results, recent studies suggest that soluble factors and EVs affect different aspects of the regeneration process and that these two fractions might act in a synergistic manner.[54] Consequently, full MSC-CM might have superior regenerative potential compared with isolated EVs or the soluble factors.

Summary and conclusions

Over the years, MSCs have been considered fibroblast-like cells, mesenchymal stem cells, multipotent marrow stromal cells, and most recently, medicinal signalling cells. Their paracrine power resulted in a paradigm shift in the field of regenerative medicine. In the current view, tissue regeneration mediated by MSCs does not depend on their differentiation capacity but rather on the release of soluble factors and extracellular vesicles, which together facilitate the endogenous regeneration process. This represents an exciting clinical and commercial avenue. The use of the secretome to substitute stem cell transplantation has a significantly lower risk profile (no rejection, no tumour formation) and is more cost-efficient, but also poses several challenges. The MSC-secretome is a biological with a poorly defined composition making regulatory affairs very challenging. In addition, there is high need of standardisation of the production process and the development of appropriate bioactivity assays to test the anti-inflammatory, immunomodulatory and proangiogenic potential.

Moving forward in developing this strategy, optimal culture conditions, sources of MSCs and pre-conditioning of cells prior to harvesting the CM need to be explored for an effective therapeutic design that can be commercially exploited.

BIOGRAPHY

DR DARIUS WIDERA is a Lecturer in Stem Cell Biology and Regenerative Medicine at the University of Reading. His lab is interested in stem cell research and the influence of injury and inflammation on somatic cells. His team wants to understand how injury signals influence differentiation, proliferation, and migration of stem cells. They also study various adult human stem cell populations and their secretome, develop 3D cell culture methods, and work on novel stem cell-based but cell-free therapies for degenerative disorders and conditions.

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