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Review

The dynamic stem cell microenvironment is orchestrated by microvesicle-mediated transfer of genetic information

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Summary. It has been commonly supposed that adult stem cells co-localize with supporting cells within specific regions or specialized microenvironment in each tissue/organ, called stem cell niche. This concept was based on the assumption that stem cells are intrinsically hierarchical in nature. However, recent data indicate that stem cells may represent a continuum with reversible alterations in phenotype taking place during the transit through cell cycle. Based on this dynamic interpretation it has been suggested that the so-called niche is represented by a single or only few cell types continually adjusting their phenotype and function to individual circumstances. A critical component in the regulation of the continuum of stem cell phenotypes is the microenvironment. In this context, microvesicles (MVs) account for the transfer of genetic information between cells. Originally considered inert cellular debris, MVs are increasingly recognized to be important mediators of cell-to-cell communication. MVs may transfer receptors, proteins, mRNA and microRNA to target cells via specific receptor-mediated interaction. In stem cell biology the exchange of genetic information may be bidirectional from stromal to stem cells. In the context of tissue injury the MV-mediated transfer of genetic information may reprogram the phenotype of stem cells to acquire features of the injured tissue cells. In addition, MVs derived from stem cells may induce de-differentiation of cells which have survived injury with a cell cycle re-entry that may allow tissue regeneration. In the present review we discuss the possibility of a continuous genetic modulation of stem cells by a MV-mediated transfer of information between cells.

Key words: Stem cells, Microvesicles, Stem cell niche

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Introduction

Stem cells are a subset of cells capable of unlimited self renewal and of high multilineage differentiation potential into different types of mature cells. Based on these characteristics, stem cells play essential roles in organogenesis during embryonic development, and in the adult are responsible for the growth, homeostasis and repair of many tissues.

Stem cells may be classified according to their origin and developmental status in embryonic and adult stem cells. The embryonic stem cells (ESC) are derived from the inner cell mass of the blastocyst-stage mammalian embryo a few days after fertilization and they are pluripotent, giving rise to the germ line during development and to virtually all tissues of the organism.

In contrast to ESC, the stem cells present in the adult organism (tissue-resident adult stem cells) are undifferentiated cells localized in differentiated tissues, with a more limited self renewal and differentiation potential, usually restricted to cell types of the tissue from which they originate. The adult stem cells are involved in tissue homeostasis and repair after wounding over the lifetime. Many studies indicate that in pathological conditions adult stem cells can actively participate in tissue cell replenishment after being in a quiescent state for short or long periods of time (Bryder et al., 2006; Mimeault and Batra, 2006; Mimeault et al., 2007) These cells when partially committed to differentiate in a defined lineage are also named progenitor cells. Adult stem/progenitor cells have been identified in most tissues and organs of mammalian organisms, such as bone marrow, liver, pancreas, heart, kidney, brain, lung, digestive tract, retina, breast, ovaries, prostate, testis, dental pulp, hair follicles, skin, skeletal muscle, adipose tissue and blood (Mimeault and Batra, 2008).

In tissues that require a high cell turnover, such as the hematopoietic system, the intestine and the skin,

stem cells are critical for maintaining their homeostasis. In other tissues with a much lower rate of cell turnover, such as the kidney, the lung, skeletal muscle and the liver, resident stem cells may awake following injury and participate in tissue repair.

The most common source of adult stem cells is the bone marrow, where there are two main stem cell populations: the hematopoietic stem cells (HSC), committed to differentiate into fully specialized cells of the blood (erythrocytes, thrombocytes and leukocytes), and the mesenchymal stem cells (MSC), undifferentiated adult stem cells of mesodermal origin that have the capacity to differentiate into cells of connective tissue lineages, including bone, fat, cartilage and muscle (Jiang et al., 2002). Moreover, resident adult stem cells have been isolated from several tissues, including the central nervous system (Reynolds and Weiss, 1992), retina (Tropepe et al., 2000), skeletal muscle (Jackson et al., 1999), liver (Herrera et al., 2006) and kidney (Bussolati et al., 2005).

Tissue resident stem cells preferentially generate differentiated cells of the tissue of origin, suggesting a relevant role in the postnatal growth of organs, in physiological turnover and in tissue repair.

The stem cell niche

It has been generally reported that tissue-resident adult stem cells are co-localized with supporting cells within specific regions or specialized micro-environments in each tissue/organ, called stem cell niche (Li and Xie, 2005; Moore and Lemischka, 2006; Jones and Wagers, 2008). In adult bone marrow the HSC could reside in the endosteal niche, associated with the osteoblasts of the inner surface of the cavities of trabecular bone that could provide factors able to regulate number and function of HSC through the secretion of osteopontin, and a complex and not completely identified paracrine signalling network i.e. Kit/Kit ligand, Notch signaling, angiopoietin-1/Tie-2 (Mitsiadis et al., 2007). Functional regulation of HSC may occur at the endosteal niche through the activation of osteoclasts by RANKL, produced by osteoblasts and mobilization of HSC into the circulation (Kollet et al., 2006). Another niche for HSC is the perivascular area of sinusoids that could provide homeostatic blood cell production and response to hematological stresses (Kiel et al., 2005). The perivascular niche is possibly the main site of stem cells in organs that do not contain bone, such as liver and spleen (Mitsiadis et al., 2007). Even in bone marrow it is considered that two thirds of HSC are localized in perivascular areas (Crisan et al., 2008; da Silva Meirelles et al., 2008; Caplan, 2009). The fenestrations of bone marrow sinusoids and the expression by the endothelial cells of chemokines and adhesion molecules such as E-selectin and VCAM-1, may be critical for HSC homing and mobilization (Sipkins et al., 2005; Wilson and Trumpp, 2006). The two niches could be functionally distinct: the endosteal niche could maintain HSC quiescence over the long term, whereas the perivascular niche could maintain HSC over a shorter time period, supporting HSC proliferation, favouring myeloid and megakaryocytic lineage differentiation and mediating HSC entry into circulation (Mitsiadis et al., 2007; Perry and Li, 2007). The other stem cells present in bone marrow are MSC, which play a role in providing stromal support for HSC in the bone marrow (Noort et al., 2002). MSC localize in perivascular areas in the bone marrow in close association with HSC (Shi and Gronthos, 2003) and could play a role in maintaining quiescence of HSC by inhibiting their proliferation and differentiation (Glennie et al., 2005).

In search of a tissue resident stem cell niche

Many studies reported the presence of other stem cell niches in mammals (Li and Xie, 2005; Da Silva Meirelles et al., 2008): the epithelial stem cell niche in skin resides in the bulge area of the hair follicle beneath the sebaceus gland (Cotsarelis et al., 1990; Sun et al., 1991; Niemann and Watt, 2002); the intestinal stem cell niche, where stem cells are located at the fourth or fifth position above the Paneth cells from the crypt bottom (Booth and Potten, 2000; He et al., 2004; Sancho et al., 2004); finally, the neural stem cell niche at the subventricular zone and the subgranular zone of the hippocampus where neural stem cells could reside and support neurogenesis in the adult brain (Doetsch et al., 1999; Temple, 2001).

Several studies suggest that the adult tissue resident stem cells belong to the MSC lineage (da Silva Meirelles et al., 2006). However, MSC derived from different organs are defined mainly by functional *in vitro* assays using cultured cells, and their *in vivo* exact localization and function remain elusive (da Silva Meizelles et al., 2008).

The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposed the minimal criteria to define human MSC (Dominici et al., 2006; Da Silva Meirelles et al., 2008), which include cell positivity for CD105, CD73, and CD90 and negativity for CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA-DR. However, a specific identification marker for isolation of MSC or their identification in tissues is still lacking. Stro-1 marker is not exclusively expressed by MSC and may be lost during culture (Kolf et al., 2007). Recent studies have shown that stage-specific embryonic antigen 1 (SSEA-1) and SSEA-4 are expressed by human bone marrow MSC (Anjos-Afonso and Bonnet, 2007; Gang et al., 2007). Moreover, CD200 has been suggested as a marker useful for the purification of bone marrow MSC, although it is also expressed by thymocytes, B and T lymphocytes, and endothelial cells (Wright et al., 2001), if used in combination with other MSC markers. The minimal criteria to define human MSC include also osteo-, chondro-, and adipogenic differentiation capabilities.

However, in vitro results have not allowed much insight into the recognition of these cells in organs. Two different possibilities are considered to date. First, the possibility that MSC are only located in the bone marrow, from which they may circulate to other tissues to replenish losses of cell population following physiological turnover or tissue injury. Against this hypothesis stands the considerable difficulty in establishing MSC cultures from peripheral blood (Wexler et al 2003; da Silva Meirelles et al., 2006). As an alternative, the possibility that different tissue-intrinsic stem cells might behave *in vitro* as MSC since MSC have been isolated from virtually all different tissues with very similar morphologic, immuno-phenotypic, and functional properties (da Silva Meirelles et al., 2006). In this context, a perivascular location for MSC has been suggested, correlating these cells with pericytes that would adequately explain why MSC can be isolated from all tissues. The establishment of MSC-like cultures from isolated blood vessels, including decapsulated glomeruli (da Silva Meirelles et al., 2006), argues in favour of this hypothesis. In this model the perivascular zone may act as a MSC niche *in vivo*, where microenvironment factors may modulate their phenotype with transition to progenitor and mature cells.

Stem cell niche or area of influence?

For many years it has been assumed that the regulation of bone marrow stem cells was intrinsically hierarchical in nature with a progressive loss of proliferative potential and expression of differentiated phenotypes (Till et al., 1964). More recently, a continuum model of stem cell biology has been proposed (Colvin et al., 2004; Quesenberry et al., 2005). On the basis of the well-known stem cell plasticity, stem cells represent a very heterogeneous population. It has been postulated that the phenotype of bone marrow stem cells is labile, it varies with position in the cell cycle and that it is reversible (Fig. 1). Therefore, bone marrow stem cells may be defined not at a single cell level, because the single cell phenotype and functional potential continually and reversibly change, but rather on a population basis (Colvin et al., 2007). This cell-cycle reversibility of hematopoietic and non hematopoietic cells is at the basis of the continuum model of stem cell biology, in which the phenotype of stem cells is reversibly changing during the cell cycle transit until a terminal-differentiating stimulus is encountered at a cycle-susceptible time (Colvin et al., 2007). In this model the status of the cell cycle and the exposure to products from injured cells may play key roles in determining the differentiated expression of a bone marrow stem cell. The same stem cell may show different phenotypes in different functional states, possibly tied up to cell cycle phase (Quesenberry et al., 2007). Recently, Quesenberry and Aliotta (2008) proposed the existence not simply of several unique niche cells, but of a variety of niche cells which continually change phenotype to appropriately interact with the continuum of stem cell phenotypes. Since bone marrow stem cells continually change their phenotype and are very heterogeneous, there exist innumerable different niche cells. These Authors are doubtful that the so-called niche is represented by a single or only a few cell types, and prefer to designate the bone marrow microenvironment as consisting of areas of influence which are continually adjusting to individual circumstances (Quesenberry and Aliotta, 2008). This concept may possibly be extended to tissue resident stem cells.

Stem cell plasticity and microenvironment interactions

Several studies have confirmed the plasticity of bone marrow stem cells leading to differentiation in non-hematopoietic cells, although it is not clear whether transdifferentiation, dedifferentiation or fusion occur (Quesenberry et al., 2007). Endogenous bone marrow-derived stem cells have been reported to contribute to the repair of tissue injury. Some reports indicate that bone marrow derived stem cells are capable of engraftment into damaged tissues, although the lineage of stem cells recruited has not been definitively established. Based on bone marrow transplantation in mouse Fang et al. (2008) demonstrated that bone marrow derived cells contribute to the repair of acute

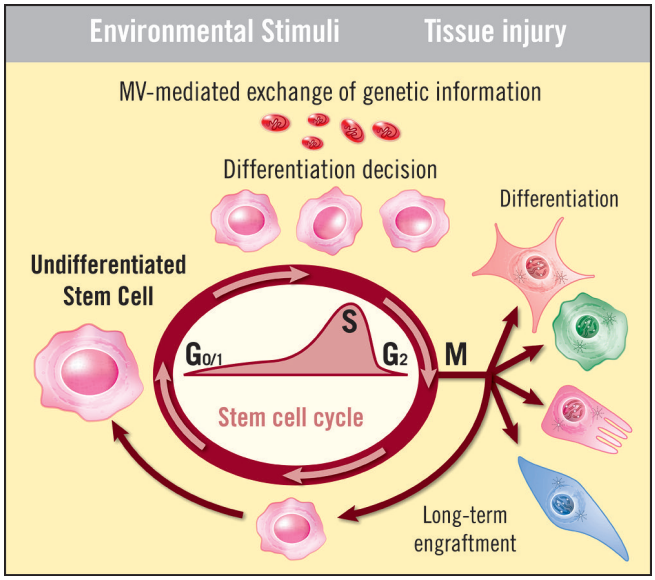

Fig. 1. Schematic representation of the Continuum model of stem cell differentiation. In this model the phenotype of stem cells is reversibly changing during the cell cycle transit until a terminal-differentiating stimulus is encountered (Quesenberry et al., 2007). The stem cell phenotype is very flexible, depending on cell cycle phase and specific microenvironment and could characterize the same cell in different functional states or phases of cell cycle.

renal injury and suggested that the HSC rather than the MSC are involved.

However, this issue remains highly controversial and several studies point to a paracrine/endocrine action of endogenous stem cells rather than of a direct repopulation of the injured tissues (Humphreys and Bonventre, 2008). Experiments with exogenous MSC administration in AKI demonstrate a functional and morphologic recovery associated only with a transient recruitment of MSC within the renal vasculature with a minimal incorporation within the regenerating tubules (Duffield et al., 2005; Tögel et al., 2005). It has been suggested that the transient presence of the MSC within the injured tissues may provide a paracrine support to the repair, which is mainly sustained by intrinsic epithelial cells which survived injury. (Humphreys et al., 2008) Indeed, there is a growing body of evidence supporting the hypothesis that paracrine/endocrine mechanisms mediated by factors released by the bone marrow-derived stem cells play an essential role in the repair observed after stem cell mobilization or injection into injured kidney or infarcted hearts (Gnecchi et al., 2008).

Strong support of a paracrine/endocrine mechanism for tissue repair comes from experiments of administration of conditioned medium from MSC, which was found to be able to mimic the beneficial effects of stem cell therapy. Bi et al. (2007) showed that MSC may protect kidney from toxic injury independently from their tubular engraftment, by producing factors that limit apoptosis and enhance proliferation of tubular cells. Similar results were obtained for the beneficial effect of stem cells in myocardial injury (Gnecchi et al., 2008). The frequency of stem cell engraftment and the number of newly generated cardiomyocytes and vascular cells, either by transdifferentiation or cell fusion, appear too low to explain the beneficial effects observed (Gnecchi et al., 2008). On the other hand several studies indicate that stem cell released soluble factors may contribute to cardiac repair and regeneration (Caplan and Dennis, 2006). One can also postulate that paracrine factors may mediate endogenous regeneration via activation of resident cardiac or kidney stem cells. Therefore, a dynamic regulation and interaction of stem cell derived factors that influence cell survival and tissue regeneration can be proposed, involving the activation of resident and circulating stem cells. Specific studies will need to define the factors and the pathways involved, as well as their temporal and spatial expression in the injured tissues (Gnecchi et al., 2008). A comprehensive characterization of the paracrine factors and their pathways will allow a better understanding of stem cell biology and the identification of new therapeutic strategies.

Besides soluble factors, microvesicles (MVs) have been described as a new mechanism of cell to cell communication, potentially involved in the stem cell biological actions (Morel et al., 2004; Ratajczak et al., 2006a).

Role of microvesicles in cell to cell communication

MVs are derived from the endosomal membrane compartment after fusion with the plasma membrane and are released from the cell surface of activated cells (Heijnen et al., 1999; Rozmyslowicz et al., 2003). MVs are shed by various cell types, such as circulating blood cells and cells of the vessel wall during cell activation by agonists and physical or chemical stress. In vivo, the majority of MVs are derived from platelets (George et al., 1982), and to a lesser extent from other blood cells and endothelial cells (Martinez et al., 2005). MVs represent a heterogeneous population, differing in cellular origin, number, size and antigenic composition (Diamant et al., 2004). MVs were originally considered to be inert cellular debris. It is now recognized that MVs may interact with cells through specific receptor-ligand interactions, leading to target cell stimulation directly or by transferring surface receptors (Janowska-Wieczorek et al., 2001; Morel et al., 2004). MVs derived from activated platelets are able to induce metastasis and angiogenesis in lung cancer (Janowska-Wieczorek et al 2005). Moreover, tumor-derived MVs may transfer surface determinants and mRNA of tumor cells to monocytes (Baj-Krzyworzeka et al., 2006). It has been also postulated that MVs may contribute in spreading certain infective agents, such as HIV or prions (Facler and Peterlin, 2000; Fevrier et al., 2004).

The recent finding that MVs may shuttle selected patterns of mRNA and of microRNA suggests that MVs may represent a new mechanism of genetic exchange between cells (Ratajczak et al., 2006b; Deregibus et al., 2007; Dooner et al., 2008a; Yuan et al., 2009).

We demonstrated that MVs are vehicles for mRNA transport and exchange of genetic code between cells. MVs generated from endothelial progenitor cells (EPC) are incorporated in normal endothelial cells by interaction with α4- and ß1-integrins expressed on their surface (Deregibus et al., 2007). Once incorporated, MVs activate an angiogenic program in the endothelial cells by promoting cell survival, proliferation and organization *in vitro* in capillary-like structures. *In vivo*, in SCID mice, MVs stimulate human endothelial cells to organize in a patent vessel network connected with the murine vasculature. When MVs are pretreated with RNase, they lose the angiogenic activity even though they are internalized by endothelial cells. This suggests that the angiogenic effect of MVs is related to transfer of RNA following MV incorporation within the endothelial cells. The microarray analysis and the quantitative RT-PCR of MV-mRNA extract indicate that MVs derived from EPC are shuttling a specific subset of cellular mRNA, such as mRNA associated with the PI3K/AKT and eNOS signalling pathway. Protein expression and functional studies demonstrated that PI3K and eNOS play a critical role in the angiogenic effect of MVs. As proof of the effective transfer of mRNA, green fluorescence protein (GFP) can be transduced in target endothelial cells by MVs carrying GFP mRNA

(Deregibus et al., 2007). Besides mRNA, MVs may transfer in target cells microRNA, as recently shown for ESC-derived MVs (Yuan et al., 2009). Indeed, the ESC represent an abundant source of MVs and it has been suggested that MVs derived from these cells may represent one of the critical components supporting self renewal and expansion of stem cells (Ratajczak et al., 2006a,b). Moreover, Ratajczak et al. (2006b) demonstrated that ESC-derived MVs are able to reprogram hematopoietic progenitors by a horizontal transfer of mRNA and protein delivery. Taken together these results indicate that MVs may be important paracrine mediators of signaling within stem cells and differentiated cells by transferring selected patterns of proteins, mRNA and microRNA.

Role of MVs derived from injured tissue in inducing change in the phenotype of bone marrow stem cells

The evidence that MVs may act as paracrine mediators raises the question as to whether they could be involved in injured tissue-induced change in the phenotype of stem cells. Several studies indicate that bone marrow derived stem cells may contribute to tissue repair. It has now been established that bone marrow cells, probably the HSC which are mobilized in higher numbers than MSC, have the capacity to produce non-marrow cells in many injured tissues after engraftment (Quesenberry et al., 2007, Dooner et al., 2008b). The underlining mechanism leading to stem cell activation/differentiation after interaction with injured cells remains to be defined. Transdifferentiation as a mechanism of stem cell plasticity is highly unlikely, since it has never been conclusively demonstrated in any experimental setting (Quesenberry et al., 2007). Fusion appears to be a mechanism of marrow plasticity in some reports but not in others (Jang et al., 2004a; Rovó and Gratwohl, 2008; Colletti et al., 2009). In lung injury, cell fusion has been evaluated in cross-sex transplantation experiments but did not explain the lung differentiation of bone marrow-derived stem cells (Harris et al., 2004). On the other hand, differentiation from a rare population of resident stem cells has never been excluded. Epigenetic cell changes mediated by signals received from injured cells are possibly involved in stem cell differentiation. Quesenberry et al. (2008) suggested that differentiation in response to specific signals, especially from injured cells, may be delivered in unique fashion by MV-mediated transfer of genetic information (Fig. 2A). Jang and Sharkis (2004b) demonstrated in co-culture experiments of bone marrow cells, separated from injured liver cells by a cell impermeable membrane, that bone marrow cells express the albumin gene and the protein. Dooner et al. (2008a), in a similar experimental setting, demonstrated that murine bone marrow cells express genes for lung-specific proteins such as Clara cell-specific protein, surfactant B, and surfactant C. Moreover, they found that the injured lung conditioned medium also induced lung-specific gene expression in bone marrow cells and that this activity resided in MVs released in the cell supernatant. These MVs were shown to contain high levels of lung-specific mRNA and to deliver this mRNA to bone marrow cells, suggesting that MV derived from injured tissue might mediate bone marrow cell phenotype change during physiologic tissue repair.

Regenerative potential of microvesicles

On the other hand, MVs released from stem cells

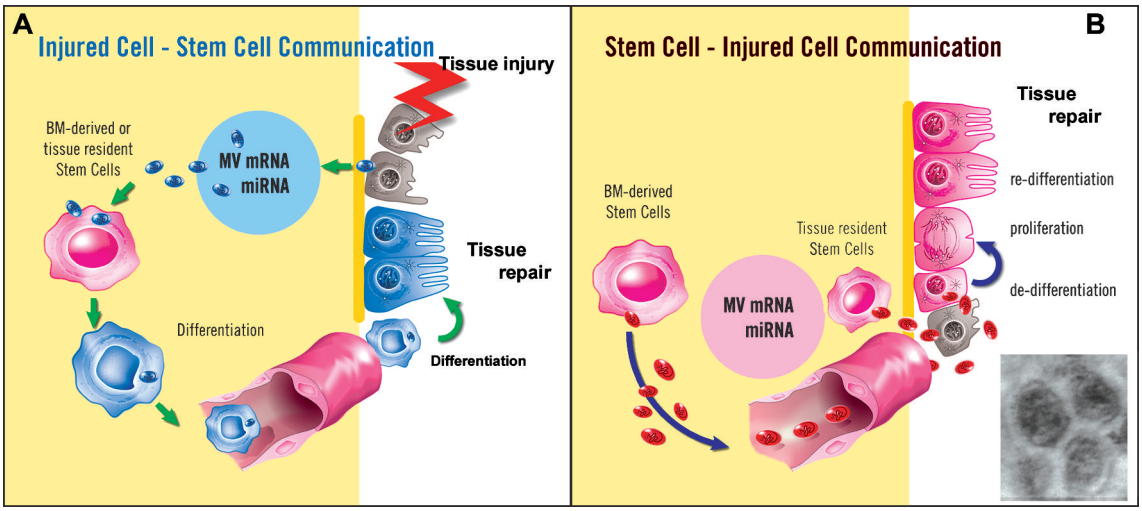

Fig. 2. Schematic representation of MV-mediated mechanism of tissue repair. The MV-mediated exchange of genetic information may be bidirectional. **A.** MVs derived from injured cells may transfer to stem cells specific subsets of mRNA and microRNA that induce a stem cell differentiation with a contribution to the repair of the injured tissue. **B.** MV-mediated transfer of mRNA, micro RNA or proteins from bone marrow-derived or tissue resident stem cells may reprogram the phenotype of mature cells by inducing de-differentiation of cells which survived injury and their re-entry into the cell cycle, with repopulation of the injured tissue and re-differentiation into mature tissue cells. Inset: micrograph of transmission electron microscopy of MVs released from human MSC. The image shows small vesicles of approximately 120 nm in diameter. (Modified from Camussi et al. (2009).

recruited at the site of tissue injury may induce de-differentiation of resident cells which survived injury, making them transiently acquire a stem cell-like phenotype with the activation of tissue regenerative programs (Fig. 2B). In this context one can interpret the MV-induced activation of a pro-angiogenic program in endothelial cells (Deregibus et al., 2007). Moreover, we recently demonstrated that MVs derived from human MSC are able to stimulate *in vitro* proliferation and apoptosis resistance of tubular epithelial cells (Bruno et al., 2009). When administered *in vivo* in SCID mice with acute kidney injury MVs accelerate the functional and morphological recovery. The beneficial effect of MV administration is comparable to that of human bone marrow-derived MSC. Also, in this experimental setting RNase treatment of MVs abrogate both the *in vitro* and the *in vivo* effects of MVs, suggesting that mRNA shuttled by MVs is the final effector of their biological activity. Differently from MVs derived from EPC, where we identified mRNA for defined signal transduction pathways, MVs released by MSC contain mRNA representative of the multiple differentiative and functional properties of MSC, indicating a cell specificity of the mRNA content. The effective horizontal transfer of mRNA is indicated by the presence of human specific mRNA and proteins in tubular cells of mice with acute kidney injury treated with MVs derived from human MSC.

The mechanisms involved in tissue regeneration induced by the administration of exogenous stem cells are still unclear. Our own bias is toward an MV-mediated transfer of mRNA/proteins derived from stem cells that may induce de-differentiation of mature cells, triggering a proliferative program that may contribute to the repair of tissue injury.

These studies open new research perspectives on the use of MVs to transfer RNA-based information from stem cells/precursors to injured cells as a potential therapeutic strategy. An advantage of using MVs in regenerative medicine instead of stem cells is to avoid the possible maldifferentiation of engrafted cells that may occur in the long term (Kunter et al., 2007).

Conclusion

Recent work by several groups has suggested a basic mechanism for cell plasticity based on the exchange of genetic material, which may involve either differentiation of stem cells or de-differentiation of mature cells. This mechanism might underlie the observed functional changes in target cells and corroborate the continuum theory of stem cell biology. According to this theory the stem cells continuously and reversibly change their phenotype on a cell cycle–related manner. This would imply that various stem cell types may be simply functional variants of one another.

Studies showing that MVs mediate transfer of genetic information suggest a unique mechanism for the plasticity that could explain the observed plasticity of stem cells and also the functional effects without the need of transdifferentiation into tissue cells. The MV-mediated transfer of genetic information mimics a mini-cell fusion with deep effects on the phenotype of stem cells (Quesenberry and Aliotta, 2008). On the other hand, MVs derived from stem cells may re-direct altered functions in target cells, suggesting that they could be exploited in regenerative medicine to repair damaged tissues (Camussi et al., 2009).

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