Extracellular vesicles of mesenchymal stem or stromal cells

From bench to bedside

Bernd Giebel, Verena Börger, Mario Gimona, Eva Rohde

With the beginning of the new millennium, great hopes were placed in the rapidly developing field of stem cell research. It was and is the goal to develop stem cell therapies that help to successfully treat a wide range of degenerative diseases as well as more acute diseases such as stroke or myocardial infarction. Conceptually, it was assumed that injected stem cells or their descendants migrate into affected tissues and replace lost cell types via transdifferentiation, thus alleviating disease associated symptoms. At that time, human embryonic stem cells came into focus of scientific interest. However, due to their high teratogenic potential and ethical explosiveness they could not be used for stem cell therapies. Instead, various somatic stem cell types were considered and used as therapeutic agents.

Keywords: *MSCs – stem cell therapy – extracellular vesicles – exosomes – immunomodulation – cell-free therapy*

MSCs

Especially fibroblastoid cells that can easily be raised from bone marrow and other tissues (including fat and umbilical cord) and that were initially described by Friedenstein and colleagues in the 1960s [1], became the therapeutic cell source of choice in a still increasing number of clinical trials. Such cells display high proliferation potential and lack teratogenic potential. Since these cells were able to differentiate into adipogenic, chondrogenic and osteogenic cell types, (which are considered as mesodermal derivatives), they were initially referred to as mesenchymal stem cells (MSCs) [2]. Relying on their therapeutic potential and the fact that their use in animal models produced no recognizable side effects [3], MSCs were then also used successfully in initial therapeutic trials. The question quickly arose as to how the immune system responds to the application of donor MSCs. While it was initially thought that MSCs are rejected in principle, it has been shown that MSCs can modulate the activity of different types of immune cells in patients. They very efficiently suppress immune effector responses and propagate regulatory immune responses, that is, they switch the immune system from the defense to the tolerance state [4-6]. In addition to the regenerative potential of MSCs, their immunotherapeutic activity has been tested in the clinic [7]. To date, nearly 1,000 clinical trials have been registered at the National Institute of Health (NIH) in, which MSCs had been or will be used to treat a wide variety of different diseases (www.Clinicaltrials.gov).

Although the outcome of several clinical studies appears controversial, many studies show therapeutic effects of applied MSCs in at least some patients. Ongoing studies that investigated the bio-distribution of injected or infused MSCs *in vivo* have shown that most of the cells end up

in the lungs and only occasionally are found in the region of the intended target tissue. Attempts to clarify whether the cells need to migrate into affected tissues to achieve their therapeutic functions demonstrated that in most cases MSCs act in a paracrine rather than a cellular manner [8, 9]. The differentiation potential of MSCs, which sometimes was regarded as pluripotent, has also been questioned experimentally. Consequently, today, many scientists question the stem cell character of MSCs. To keep the abbreviation MSC, these cells are now increasingly referred to as *mesenchymal stromal cells*; also the term *medical signaling cells* has been suggested by a leading MSC researcher [10].

Whatever MSCs may be called in the future, many scientists have tried to identify the active therapeutic substance(s) that they release into their environment. In 2009, at the example of an acute renal damage model and in 2010, at the example of a myocardial infarction model, and by using different preparation methods, two groups demonstrated that the active component is located in fractions of processed culture supernatant that contain high concentrations of vesicular structures. At that time these vesicles were called microvesicles or exosomes, respectively; today, one would correctly refer to them as extracellular vesicles (EVs) [11, 12].

Extracellular vesicles

As described in article 1 of this issue, EVs are entities that mediate intercellular communication over long distances, EV are delivered by different cells and are detectable in all bodily fluids [13]. They are composed of a heterogeneous collection of lipids, proteins and RNAs. As non-self-replicating units that according to their small size (70-150 nm) can be sterilized by filtration, small EVs have in principle significant advantages over cells for therapeutic applications.

MSC-EVs

Indeed, EVs derived from MSC culture supernatants have been already successfully used in an individual treatment attempt of a steroid refractory graft-versus-host disease (GvHD) patient and in a clinical trial to treat chronic kidney disease [14, 15]. In both settings promising therapeutic effects have been observed without any reported or detected side effects. More recently, a first-in-man approach of topical MSC-EV administration has been performed to improve the implantation-induced injury that occurs during the surgical procedure of cochlea electrode insertion. Like in the other two applications no adverse reactions were recorded, however, the hearing capability of the treated patient was significantly improved (Warnecke, Gimona, Rohde et al., in preparation).

Irrespective of how MSC-EVs have assisted in improving the symptoms in aforementioned patients, their therapeutic potential is also documented in an increasing amount of different preclinical models. In addition to the initial reports on the therapeutic potential of MSC-EVs in the acute renal damage and cardiac infarction models, positive effects of MSC-EVs were confirmed in acute and chronic renal damage models by independent groups [16-18]. Positive effects have also been reported on liver, lung and muscle regeneration [19-21]. Furthermore, MSC-EVs were found to promote blood circulation in a rat model of critical limb ischemia, the healing of skin burns, and the survival of allogeneic skin grafts [22-24]. Within the nervous system, positive effects were observed on ischemic stroke symptoms in rat and mouse models as well as on the regeneration of sciatic nerves in rats [25-27].

MSC-EVs can act via different mechanisms to improve the symptoms of respective diseases. Their exact modes of action have not been unraveled, yet. However, it appears that their capability to modulate immune responses and switch the immune system from the acute inflammatory into its regulatory state, i.e. to switch from defense to tolerance, is one of their key-functions [28, 29]. An important aspect in translating MSC-EVs into the clinic is the consideration that individual MSC-EV preparation may vary in their therapeutic effectiveness. To this end, MSCs have already been recognized as a heterogeneous cell entity. Independent of their origin, MSCs from given sources can differ in size and in the expression level of *bona fide* MSC cell surface antigens [30, 31]. Accordingly, it can be assumed that there are different MSC subtypes, which very likely differ in their therapeutic potentials. In this context, we are not aware of any generally accepted criteria to discriminate different MSC subtypes. In line with the postulated functional heterogeneity of MSCs many groups reported positive clinical effects following MSC administration, however, there are also several reports which could not reproduce observed effects [32]. Indeed, a phase III clinical trial in which GvHD patients were treated with MSCs ("Osiris Study", NCT00366145), failed to show efficacy [33]. Thus, to avoid such drawbacks in the MSC-EV field it is man-

datory to define criteria to discriminate therapeutic active from less active and nonactive MSC-EV preparations. For now, it is one of the mayor challenges of the field to set up functional assays which reflect the therapeutic potency of MSC-EV preparations.

Furthermore, MSC-EVs need to be manufactured under GMP-compliant conditions, preferably in a scalable manner. Due to the novelty of the field, however, there are still some technical hurdles. So far, there are no standardized procedures to prepare EVs in larger quantities. Furthermore, there is a lack of qualified techniques to study EVs at the single-particle level similar to cells using flow cytometry. As device manufacturers and the pharmaceutical industry become increasingly aware of the potential of the EV field, we expect that preparative and analytical methods will improve significantly in the coming years. Also, the regulatory requirements, which for now have been formulated by only some national regulatory authorities, might get harmonized. We are involved in international activities to promote MSC-EVs and other EV products effectively into the clinics, and have published a number of manuscripts discussing the current state of the art in more details as well as strategies to address challenges in the translational field [34-36].

Summary and Résumé

MSCs have been and are widely used in regenerative and immunotherapies. Recent findings suggest that their therapeutic effect is mediated, at least in part, by EVs. Because EVs can be sterilized by filtration and do not replicate themselves, EV therapies offer significant advantages over cell therapies. It appears that MSC-EVs mediate immunosuppressive functions and promote angiogenesis; direct influences on somatic stem cells can be assumed. Even though no side effects have been described so far in the preclinical models or in the individual therapeutic treatment's attempts, safety evaluation in early clinical trials are needed to find or exclude pro-tumorigenic, immunologic or other potentially harmful adverse events. The future will show whether MSC-EVs can affirm themselves as safe and potent cell-free therapeutics.

Literatur

1. Friedenstein, A.J., et al., Heterotopic of bone marrow. Analysis of precursor cells for osteogenic and hematopoietic tissues. Transplantation, 1968. 6(2): p. 230-47.

2. Pittenger, M.F., et al., Multilineage potential of adult human mesenchymal stem cells. Science, 1999. 284(5411): p. 143-7.

3. Prockop, D.J., et al., Evolving paradigms for repair of tissues by adult stem/progenitor cells (MSCs). J Cell Mol Med, 2010. 14(9): p. 2190-9.

4. Di Nicola, M., et al., Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. Blood, 2002. 99(10): p. 3838-43.

5. Bartholomew, A., et al., Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. Experimental hematology, 2002. 30(1): p. 42-8.

6. Aggarwal, S. and M.F. Pittenger, Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood, 2005. 105(4): p. 1815-22.

7. Ghannam, S., et al., Immunosuppression by mesenchymal stem cells: mechanisms and clinical applications. Stem Cell Res Ther, 2010. 1(1): p. 2.

8. Gnecchi, M., et al., Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. Nat Med, 2005. 11(4): p. 367-8.

9. Timmers, L., et al., Reduction of myocardial infarct size by human mesenchymal stem cell conditioned medium. Stem cell research, 2007. 1(2): p. 129-37.

10. Caplan, A.I., Mesenchymal Stem Cells: Time to Change the Name! Stem Cells Transl Med, 2017. 6(6): p. 1445-1451.

11. Bruno, S., et al., Mesenchymal stem cell-derived microvesicles protect against acute tubular injury. Journal of the American Society of Nephrology : JASN, 2009. 20(5): p. 1053-67.

12. Lai, R.C., et al., Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. Stem Cell Res, 2010. 4(3): p. 214-22.

13. Ludwig, A.K. and B. Giebel, Exosomes: small vesicles participating in intercellular communication. Int J Biochem Cell Biol, 2012. 44(1): p. 11-5.

14. Kordelas, L., et al., MSC-derived exosomes: a novel tool to treat therapy-refractory graft-versus-host disease. Leukemia, 2014. 28(4): p. 970-3.

15. Nassar, W., et al., Umbilical cord mesenchymal stem cells derived extracellular vesicles can safely ameliorate the progression of chronic kidney diseases. Biomater Res, 2016. 20: p. 21.

16. Gatti, S., et al., Microvesicles derived from human adult mesenchymal stem cells protect against ischaemia-reperfusion-induced acute and chronic kidney injury. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association, 2011. 26(5): p. 1474-83.

17. Bruno, S., et al., Microvesicles derived from mesenchymal stem cells enhance survival in a lethal model of acute kidney injury. PLoS One, 2012. 7(3): p. e33115.

18. He, J., et al., Bone marrow stem cells-derived microvesicles protect against renal injury in the mouse remnant kidney model. Nephrology, 2012. 17(5): p. 493-500.

19. Li, T., et al., Exosomes derived from human umbilical cord mesenchymal stem cells alleviate liver fibrosis. Stem cells and development, 2013. 22(6): p. 845-54.

20. Lee, C., et al., Exosomes mediate the cytoprotective action of mesenchymal stromal cells on hypoxia-induced pulmonary hypertension. Circulation, 2012. 126(22): p. 2601-11.

21. Nakamura, Y., et al., Mesenchymal-stem-cell-derived exosomes accelerate skeletal muscle regeneration. FEBS Lett, 2015. 589(11): p. 1257-65.

22. Zhang, H.C., et al., Microvesicles derived from human umbilical cord mesenchymal stem cells stimulated by hypoxia promote angiogenesis both in vitro and in vivo. Stem cells and development, 2012. 21(18): p. 3289-97.

23. Zhang, B., et al., HucMSC-exosome mediated -Wnt4 signaling is required for cutaneous wound healing. Stem Cells, 2014.

24. Zhang, B., et al., Mesenchymal stem cell secretes immunologically active exosomes. Stem cells and development, 2014. 23(11): p. 1233-44.

25. Xin, H., et al., Systemic administration of exosomes released from mesenchymal stromal cells promote functional recovery and neurovascular plasticity after stroke in rats. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism, 2013. 33(11): p. 1711-5.

26. Doeppner, T.R., et al., Extracellular vesicles improve post-stroke neuroregeneration and prevent post-ischemic immunosuppression. submitted.

27. Raisi, A., et al., The mesenchymal stem cell-derived microvesicles enhance sciatic nerve regeneration in rat: a novel approach in peripheral nerve cell therapy. J Trauma Acute Care Surg, 2014. 76(4): p. 991-7.

28. Börger, V., et al., Mesenchymal Stem/Stromal Cell-Derived Extracellular Vesicles and Their Potential as Novel Immunomodulatory Therapeutic Agents. Int J Mol Sci, 2017. 18(7).

29. Giebel, B. and D.M. Hermann, Identification of the right cell sources for the production of therapeutically active extracellular vesicles in ischemic stroke. Annals of Translational Medicine, 2019.

30. Vogel, W., et al., Heterogeneity among human bone marrow-derived mesenchymal stem cells and neural progenitor cells. Haematologica, 2003. 88(2): p. 126-33.

31. Dominici, M., et al., Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy, 2006. 8(4): p. 315-7.

32. Baron, F. and R. Storb, Mensenchymal Stromal Cells: A New Tool against Graft-versus-Host Disease? Biology of blood and marrow transplantation : jour-

nal of the American Society for Blood and Marrow Transplantation, 2011.

33. Galipeau, J., The mesenchymal stromal cells dilemma--does a negative phase III trial of random donor mesenchymal stromal cells in steroid-resistant graft-versus-host disease represent a death knell or a bump in the road? Cytotherapy, 2013. 15(1): p. 2-8.

34. Lener, T., et al., Applying extracellular vesicles based therapeutics in clinical trials - an ISEV position paper. J Extracell Vesicles, 2015. 4: p. 30087.

35. Reiner, A.T., et al., Concise Review: Developing Best-Practice Models for the Therapeutic Use of Extracellular Vesicles. Stem Cells Transl Med, 2017. 6(8): p. 1730-1739.

36. Witwer, K.W., et al., Defining mesenchymal stromal cell (MSC)-derived small extracellular vesicles for therapeutic applications. J Extracell Vesicles, 2019. 8(1): p. 1609206.

Prof. Dr. rer. nat. Bernd Giebel[1]
Institut für Transfusionsmedizin
Universitätsklinikum Essen
Virchowstr. 179
D-45147 Essen
Tel.: 0201-7234204
bernd.giebel@uk-essen.de
www.uk-essen.de

Verena Börger[1]

Mario Gimona[2,3,4]

Eva Rohde[2,3]

[1]Institut für Transfusionsmedizin,
Universitätsklinikum Essen,
Universität Duisburg-Essen,
Essen, Deutschland

[2]Universitätsinstitut für Transfusionsmedizin,
Universitätsklinikum der Paracelsus
Medizinischen Privatuniversität,
Salzburg, Österreich

[3]Spinal Cord Injury and Tissue Regeneration
Center Salzburg (SCI-TReCS) der Paracelsus
Medizinischen Privatuniversität,
Salzburg, Österreich

[4]Forschungsprogramm
„Nanovesikuläre Therapien" der Paracelsus
Medizinischen Privatuniversität,
Salzburg, Österreich