

Hypothesis

*Corresponding author

Yingjian Zhu, PhD, MD

Department of Urology

Shanghai First People's Hospital

School of Medicine

Shanghai Jiao Tong University

Shanghai No 100, Haining Road,

Shanghai, PR 200080, China

Tel. 86-21-63240090-3161

Fax: 86-21-63240825

E-mail: zhuyingjian_sjtu@126.com

Volume 1 : Issue 2

Article Ref. #: 1000NPOJ1105

Article History

Received: June 3rd, 2015

Accepted: July 23rd, 2015

Published: July 30th, 2015

Citation

Xiangyu Z, Yingjian Z. A new hypothesis: the immunomodulatory effects of mesenchymal stromal cell derived extracellular vesicles in ischemic kidney injury partly through spleen. *Nephrol Open J.* 2015; 1(2): 25-29. doi: [10.17140/NPOJ-1-105](https://doi.org/10.17140/NPOJ-1-105)

Copyright

©2015 Yingjian Z. This is an open access article distributed under the Creative Commons Attribution 4.0 International License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

A New Hypothesis: The Immunomodulatory Effects of Mesenchymal Stromal Cell Derived Extracellular Vesicles in Ischemic Kidney Injury Partly through Spleen

Zou Xiangyu and Zhu Yingjian*

Department of Urology, Shanghai First People's Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, PR 200080, China

ABSTRACT

Ischemic Reperfusion Injury (IRI) is a major reason for Acute Kidney Injury (AKI) in hospitalization patients and could continue to result in end-stage kidney fibrosis. Mesenchymal Stromal Cells (MSCs) are regarded as a promising therapeutic method for AKI, but the potential ethical and tumorigenesis problems of MSCs limit clinical use. Recently Extracellular Vesicles (EVs) that contained in the MSCs' condition medium were also found having a vital therapeutic effect in IRI induced AKI. As for the pluripotent prosperities of MSCs, MSC-EVs contain various bioactive substances that participate in the tissue repair and the immunomodulatory effect of MSC-EVs has been regarded as a crucial role in ischemic kidney injury and repair. However, the true mechanism still unknown. Based on existed facts, we hypothesized that the immunomodulatory effect of MSC-EVs in ischemic kidney injury partly through spleen.

KEYWORDS: Mesenchymal stromal cell; Extracellular vesicles; Ischemic kidney injury; Immunomodulatory.

ABBREVIATIONS: IRI: Ischemic Reperfusion Injury; AKI: Acute Kidney Injury; MSCs: Mesenchymal Stromal Cells; EVs: Extracellular Vesicles; I/R: Ischemia/Reperfusion; ICAM-1: Intercellular adhesion molecule-1.

INTRODUCTION

Ischemia/Reperfusion (I/R) injury could cause the intrinsic acute kidney injury (AKI) and lead to the kidney fibrosis in later stage.¹ Viable renal cells are unable to repair the necrotic tissues due to their limited capability of regeneration, which results in the damage of renal functions. Despite the renal replacement, there is no specific therapy to improve renal function has been found in decades. These facts reveal us to investigate new strategies to treat AKI.

In recent years, the MSCs-based therapy in AKI provides us a new way to protect the renal functions. MSCs from various sources (such as bone marrow, fetal membrane and adipose) in repairing kidney injury were reported,²⁻⁴ however, the potential immune rejection, adipogenic differentiation and malignant transformation events of MSCs limit our clinical use. EVs contained in the MSC's condition medium could repair variety injured organs and also could alleviate I/R injury induced AKI.^{5,6} EVs acquire the cell surface markers when they are released by different cells and could reach the target organs in body when they were injected intravenously. Our previous studies also found human umbilical cord mesenchymal cells derived EVs reached the spleen, lungs and injured kidney in a rat AKI model and it could attenuate the ischemic kidney injury,⁷ which is consistent with the recent published results.⁸ MSC-EVs could alleviate kidney injury and protect the renal functions in the different AKI animal models,

and both the MSCs and MSC-EVs have the anti-inflammation characters *via* decreasing the pro-inflammation cells and factors in ischemic injured kidneys.^{7,9-11} The inflammation level of ischemic kidney depends on the regulation of the whole body. As one of the most important immune organs in the body, spleen has the inflammatory cell regulation functions under different stresses and it could directly affect the circulating inflammatory mediators. The inflammatory cells in the spleen have an important role in the pathophysiology of ischemic AKI, and it has been demonstrated that MSCs attenuate ischemic AKI through immunomodulatory effects in a spleen dependent manner, which means that MSCs would lose these effects without the spleen.¹² However, the possible mechanisms of MSC-EVs in IRI AKI remain unclear.

HYPOTHESIS

The effects of MSC-EVs in ischemic AKI are multiple and the immune systems have vital functions in kidney I/R injury. The immunomodulatory effects of MSC-EVs in ischemic kidney injury partly through spleen. (Figure 1).

Evaluation of the Hypothesis

The pathophysiology of ischemic AKI is complex and the role of inflammation in AKI has been well known.^{13,14} The alternation of inflammatory cells and factors in different milieu

may exert different effects. In the initiation and extension of ischemic AKI, pro-inflammation cells are increased and activated in the injured kidney. Studies have shown that T cells are the key mediators in ischemic AKI and the regulation T cells (Tregs) have the reno-protective effects.^{15,16} Pro-inflammation cells could directly kill the tubular epithelial cells or secrete pro-inflammatory factors to lead the kidney damages indirectly.¹⁷⁻²⁰ Then the inhibitions of some inflammatory pathways were used to attenuate organ I/R injury. Researchers have also found that it could significantly decrease the renal tubular apoptosis and protect the kidney functions in AKI animal models when depleting some inflammatory related cells and pro-inflammatory factors.^{21,22}

Different tissue sources acquired MSCs and MSC-EVs are used to regulate inflammatory response in injured organs. MSCs could exhibit immunosuppressive or immunomodulatory properties by inhibiting T cells and NK cells.^{23,24} The down-regulation of TNF- α , IL-6, macrophages and up-regulation of the IL-10, IL-4, Bfgf were also found after MSCs treatment in injured kidney.²⁵⁻²⁸ What is more, in the acute lung injury mice model, MSCs derived EVs reduced the neutrophils and inflammation factors both in injured lungs and plasma, and the macrophage inflammation protein-2 in the injured lungs also changed.²⁹ The infiltration of different inflammatory cells in injured kidneys depends on the cells that transmigration across the vascular endothelium in serum. As one of the most important immune organ

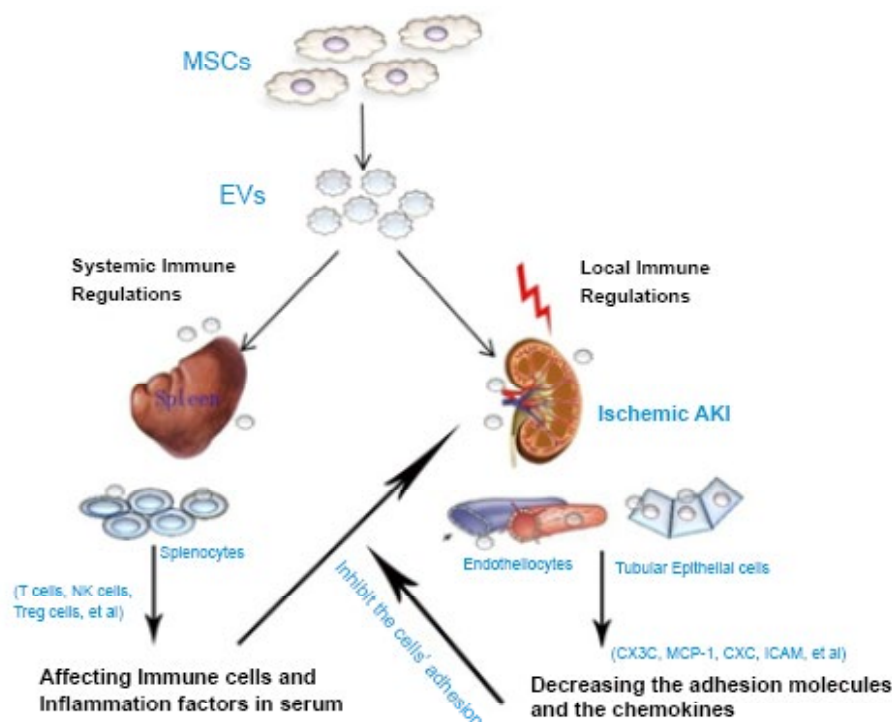


Figure 1: EVs derived from mesenchymal stromal cells have the therapy effects in AKI through accommodating both the systemic immune responses and chemokine in injured kidneys. After administration of MSC-EVs in ischemic kidney models, EVs could reach the targeted organs (spleen and injured kidneys) to change the systemic immune responses (such as change the phenotypes of T cells, NK cells, NKT cells or Tregs) and chemokine levels in injured kidneys (CX3C, ICAM-1, CXC, MCP-1, et al). Through the combined effects above, EVs ultimately lead to regulate inflammatory levels in the ischemic kidney and attenuate kidney injury.

in the body, spleen has the inflammatory cell regulation function under different stresses and it could directly affect the circulating mediators or signals. The immune cells in the spleen participate the physiopathology of AKI,^{30,31} and Jie Hu, et al. also demonstrated that MSCs attenuate AKI by immunomodulatory effects in a spleen depended manner.¹² Meanwhile, the transmigration of inflammatory cells across the vascular endothelium into kidneys depends upon various adhesion molecules, chemokine and their receptors.³² Studies have showed that a large number of chemokine like CCL2, CXCL8 are increased in kidney after ischemic AKI.³³ Moreover, activated tubular epithelial cells could express Intercellular adhesion molecule-1 (ICAM-1) and P-selectin to interact with the neutrophils, monocytes and T cells.³⁴ These factors are generated by the tubular epithelium or vascular endothelial cells in injured kidney to attract inflammatory cells. So both the immune cells in circulation systems and chemokines in injured kidneys determine the ultimate inflammation levels in injured organs.

EVs are small vesicles in the condition medium with an average about 100 nm sizes. So compared to MSCs, EVs could not only recognize the target cells *via* specific surface receptors, but also could reach various organs in body *via* circulation systems. After recognition of the target cells, EVs could change target cell phenotypes by delivering bioactive substances, such as proteins, mRNAs and miRNAs.^{35,36} Researchers have proved that endothelial cell derived EVs are able to reprogram vascular cells by transfer mRNAs and MSC-EVs protect the kidney tubular cells by transfer related miRNAs.³⁷⁻³⁹ Until now, various mRNAs in the MSC-EVs have been found, such as cell cycle related SUMO1, transcription factors related Interferon regulatory factor 6 and it also has the immune regulation related Interleukin 1 receptor antagonist and Cytokine receptor-like factor 1.³⁸ What is more, several recent reports have demonstrated that the effect of EVs is limited not only to local kidneys but to other organs,^{8,40} which may suggest the presence of a systemic effect of EVs in ischemic AKI. Based on the above facts, we hypothesize that MSC-EVs exist immunomodulatory effects in ischemic kidney injury and these effects partly through spleen.

However, there were still some questions for further detail research. First, there are many inflammatory cells in the physiopathology of ischemic AKI, any of these might be involved in EVs' therapy effects. In the previous studies, macrophages and T cells were involved in the protective role of MSCs in renal IRI.^{12,41} As for the EVs derived from MSCs, we may focus these cells for the further research. Second, the inflammation related chemokine in ischemic kidneys are multiple, such as CX3C, CXC, MCP-1, et al. To ensure the main cells and (or) factors in this process is the next works. Third, how MSC-EVs change these cell phenotypes or proteins remains controversy. Some studies showed that EVs derived from various sources could horizontal transfer nuclear acids, functional proteins, bioactive membrane and other materials to target cells.^{42,43} In our opinion any way cannot be excluded. All of these should be ex-

plicated in future research.

Consequences of the Hypothesis

Ischemic AKI is a serious condition that occurs in clinical treatment, and MSC-MVs provide the same or better therapeutic effect when compared to MSCs. In the previous studies researchers found that MSCs have the immunomodulatory effects in ischemic kidney injury in a spleen depended manner. As for the immunomodulatory effects of MSC-EVs in organ injury repair still unknown, so we hypothesize here that both systemic inflammatory cells and local kidney chemokines are regulated by MSC-EVs and these effects partly through spleen, which propose a new sight in MSC-EVs' treatment and supply the theoretical basis for the direction of clinical use.

COMPETING INTERESTS

The authors declare that they have no competing interests.

ACKNOWLEDGEMENTS

This study is supported by grants from the National Natural Science Foundation of China (81170642 and 81470919) and the fund of Shanghai First People's Hospital (12RC04).

REFERENCES

- Devarajan P. Update on mechanisms of ischemic acute kidney injury. *Journal of the American Society of Nephrology: JASN*. 2006; 17: 1503-1520. doi: [10.1681/ASN.2006010017](https://doi.org/10.1681/ASN.2006010017)
- Tsuda H, Yamahara K, Otani K, et al. Transplantation of allogenic fetal membrane-derived mesenchymal stem cells protect against ischemia-reperfusion-induced acute kidney injury. *Cell transplantation*. 2014. doi: [10.3727/096368913X665594](https://doi.org/10.3727/096368913X665594)
- Villanueva S, Carreno JE, Salazar L, et al. Human mesenchymal stem cells derived from adipose tissue reduce functional and tissue damage in a rat model of chronic renal failure. *Clin Sci (Lond)*. 2013; 125: 199-210. doi: [10.1042/CS20120644](https://doi.org/10.1042/CS20120644)
- Epicum P, Detry O, Weekers L, et al. Mesenchymal stromal cell therapy in conditions of renal ischaemia/reperfusion. *Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association - European Renal Association*. 2014. doi: [10.1093/ndt/gft538](https://doi.org/10.1093/ndt/gft538)
- Forostyak S, Jendelova P, Sykova E. The role of mesenchymal stromal cells in spinal cord injury, regenerative medicine and possible clinical applications. *Biochimie*. 2013; 95: 2257-2270. doi: [10.1016/j.biochi.2013.08.004](https://doi.org/10.1016/j.biochi.2013.08.004)
- Gatti S, Bruno S, Deregibus MC, 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: 1474-1483. doi: [10.1093/ndt/gfr015](https://doi.org/10.1093/ndt/gfr015)
7. Zou X, Zhang G, Cheng Z, et al. Microvesicles derived from human Wharton's Jelly mesenchymal stromal cells ameliorate renal ischemia-reperfusion injury in rats by suppressing CX-3CL1. *Stem cell research & therapy*. 2014; 5: 40. doi: [10.1186/scrt428](https://doi.org/10.1186/scrt428)
8. Grange C, Tapparo M, Bruno S, et al. Biodistribution of mesenchymal stem cell-derived extracellular vesicles in a model of acute kidney injury monitored by optical imaging. *International journal of molecular medicine*. 2014; 33: 1055-1063. doi: [10.3892/ijmm.2014.1663](https://doi.org/10.3892/ijmm.2014.1663)
9. Cao H1, Qian H, Xu W, et al. Mesenchymal stem cells derived from human umbilical cord ameliorate ischemia reperfusion-induced acute renal failure in rats. *Biotechnol Lett*. 2010; 32(5): 725-732. doi: [10.1007/s10529-010-0207-y](https://doi.org/10.1007/s10529-010-0207-y)
10. Liu YL, Wanga YD, Zhuanga F, Xian SL. Immunosuppression effects of bone marrow mesenchymal stem cells on renal interstitial injury in rats with unilateral ureteral obstruction. *Cell Immunol*. 2012; 276(1-2):144-52. doi: [10.1016/j.celimm.2012.05.001](https://doi.org/10.1016/j.celimm.2012.05.001)
11. Semedo P, Palasio CG, Oliveira CD, et al. Early modulation of inflammation by mesenchymal stem cell after acute kidney injury. *International immunopharmacology*. 2009; 9: 677-682. doi: [10.1016/j.intimp.2008.12.008](https://doi.org/10.1016/j.intimp.2008.12.008)
12. Hu J, Zhang L, Wang N, et al. Mesenchymal stem cells attenuate ischemic acute kidney injury by inducing regulatory T cells through splenocyte interactions. *Kidney international*. 2013; 84: 521-531. doi: [10.1038/ki.2013.114](https://doi.org/10.1038/ki.2013.114)
13. Patschan D, Patschan S, Muller GA. Inflammation and microvasculopathy in renal ischemia reperfusion injury. *Journal of transplantation*. 2012; 764154. doi: [10.1155/2012/764154](https://doi.org/10.1155/2012/764154)
14. Lee S, Huen S, Nishio H, et al. Distinct macrophage phenotypes contribute to kidney injury and repair. *Journal of the American Society of Nephrology: JASN*. 2011; 22: 317-326. doi: [10.1681/ASN.2009060615](https://doi.org/10.1681/ASN.2009060615)
15. Ascon DB, Lopez-Briones S, Liu M, et al. Phenotypic and functional characterization of kidney-infiltrating lymphocytes in renal ischemia reperfusion injury. *Journal of immunology*. 2006; 177: 3380-3387. doi: [10.4049/jimmunol.177.5.3380](https://doi.org/10.4049/jimmunol.177.5.3380)
16. Kinsey GR, Huang L, Vergis AL, Li L, Okusa MD. Regulatory T cells contribute to the protective effect of ischemic preconditioning in the kidney. *Kidney international*. 2010; 77: 771-780. doi: [10.1038/ki.2010.12](https://doi.org/10.1038/ki.2010.12)
17. Li L, Huang L, Sung SsJ, et al. NKT cell activation mediates neutrophil IFN- production and renal ischemia-reperfusion injury. *The Journal of Immunology*. 2007; 178: 5899-5911. doi: [10.4049/jimmunol.178.9.5899](https://doi.org/10.4049/jimmunol.178.9.5899)
18. Vinuesa E, Hotter G, Jung M, Herrero-Fresneda I, Torras J, Sola A. Macrophage involvement in the kidney repair phase after ischaemia/reperfusion injury. *The Journal of pathology*. 2008; 214: 104-113. doi: [10.1002/path.2259](https://doi.org/10.1002/path.2259)
19. Millar TM, Phan V, Tibbles LA. ROS generation in endothelial hypoxia and reoxygenation stimulates MAP kinase signaling and kinase-dependent neutrophil recruitment. *Free radical biology & medicine*. 2007; 42: 1165-1177. doi: [10.1016/j.freeradbiomed.2007.01.015](https://doi.org/10.1016/j.freeradbiomed.2007.01.015)
20. Zhang ZX, Wang S, Huang X, et al. NK Cells Induce Apoptosis in Tubular Epithelial Cells and Contribute to Renal Ischemia-Reperfusion Injury. *The Journal of Immunology*. 2008; 181: 7489-7498. doi: [10.4049/jimmunol.181.11.7489](https://doi.org/10.4049/jimmunol.181.11.7489)
21. Jung M, Sola A, Hughes J, et al. Infusion of IL-10-expressing cells protects against renal ischemia through induction of lipocalin-2. *Kidney international*. 2012; 81: 969-982. doi: [10.1038/ki.2011.446](https://doi.org/10.1038/ki.2011.446)
22. Lu L, Faubel S, He Z, et al. Depletion of macrophages and dendritic cells in ischemic acute kidney injury. *American journal of nephrology*. 2012; 35: 181-190. doi: [10.1159/000335582](https://doi.org/10.1159/000335582)
23. Glennie S, Soeiro I, Dyson PJ, Lam EW, Dazzi F. Bone marrow mesenchymal stem cells induce division arrest anergy of activated T cells. *Blood*. 2005; 105: 2821-2827.
24. Spaggiari GM, Capobianco A, Becchetti S, Mingari MC, Moretta L. Mesenchymal stem cell-natural killer cell interactions: evidence that activated NK cells are capable of killing MSCs, whereas MSCs can inhibit IL-2-induced NK-cell proliferation. *Blood*. 2006; 107: 1484-1490.
25. Togel F, Hu Z, Weiss K, Isaac J, Lange C, Westenfelder C. Administered mesenchymal stem cells protect against ischemic acute renal failure through differentiation-independent mechanisms. *American journal of physiology, Renal physiology*. 2005; 289: F31-F42. doi: [10.1152/ajprenal.00007.2005](https://doi.org/10.1152/ajprenal.00007.2005)
26. Eliopoulos N, Zhao J, Bouchentouf M, et al. Human marrow-derived mesenchymal stromal cells decrease cisplatin nephrotoxicity in vitro and in vivo and enhance survival of mice post-intraperitoneal injection. *American journal of physiology, Renal physiology*. 2010; 299: F1288-F1298.
27. Tsuda H, Yamahara K, Otani K, et al. Transplantation of

- allogenic fetal membrane-derived mesenchymal stem cells protects against ischemia/reperfusion-induced acute kidney injury. *Cell transplantation*. 2014; 23: 889-899. doi: [10.3727/096368913X665594](https://doi.org/10.3727/096368913X665594)
28. Luo CJ, Zhang FJ, Zhang L, et al. Mesenchymal stem cells ameliorate sepsis-associated acute kidney injury in mice. *Shock*. 2014; 41: 123-129. doi: [10.1097/SHK.0000000000000080](https://doi.org/10.1097/SHK.0000000000000080)
29. Zhu YG, Feng XM, Abbott J, et al. Human mesenchymal stem cell microvesicles for treatment of Escherichia coli endotoxin-induced acute lung injury in mice. *Stem Cells*. 2014; 32: 116-125. doi: [10.1002/stem.1504](https://doi.org/10.1002/stem.1504)
30. Kim MG, Lee SY, Ko YS, et al. CD4+ CD25+ regulatory T cells partially mediate the beneficial effects of FTY720, a sphingosine-1-phosphate analogue, during ischaemia/reperfusion-induced acute kidney injury. *Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association - European Renal Association*. 2011; 26: 111-124. doi: [10.1093/ndt/gfq480](https://doi.org/10.1093/ndt/gfq480)
31. Burne-Taney MJ, Liu M, Baldwin WM, Racusen L, Rabb H. Decreased capacity of immune cells to cause tissue injury mediates kidney ischemic preconditioning. *Journal of immunology*. 2006; 176: 7015-7020.
32. Alikhan MA, Ricardo SD. Mononuclear phagocyte system in kidney disease and repair. *Nephrology* 2012. doi: [10.1111/nep.12014](https://doi.org/10.1111/nep.12014)
33. Segerer S, Nelson PJ, Schlondorff D. Chemokines, chemokine receptors, and renal disease: from basic science to pathophysiologic and therapeutic studies. *Journal of the American Society of Nephrology: JASN*. 2000; 11: 152-176.
34. Ho AW, Wong CK, Lam CW. Tumor necrosis factor-alpha up-regulates the expression of CCL2 and adhesion molecules of human proximal tubular epithelial cells through MAPK signaling pathways. *Immunobiology*. 2008; 213: 533-544. doi: [10.1016/j.imbio.2008.01.003](https://doi.org/10.1016/j.imbio.2008.01.003)
35. Mause SF, Weber C. Microparticles: protagonists of a novel communication network for intercellular information exchange. *Circulation research*. 2010; 107: 1047-1057.
36. Morel O, Toti F, Hugel B, Freyssinet JM. Cellular microparticles: a disseminated storage pool of bioactive vascular effectors. *Current opinion in hematology*. 2004; 11: 156-164.
37. Deregibus MC, Cantaluppi V, Calogero R, et al. Endothelial progenitor cell derived microvesicles activate an angiogenic program in endothelial cells by a horizontal transfer of mRNA. *Blood*. 2007; 110: 2440-2448.
38. Bruno S, Grange C, Deregibus MC, et al. Mesenchymal stem cell-derived micro vesicles protect against acute tubular injury. *Journal of the American Society of Nephrology: JASN*. 2009; 20: 1053-1067. doi: [10.1681/ASN.2008070798](https://doi.org/10.1681/ASN.2008070798)
39. Lindoso RS, Collino F, Bruno S, et al. Extracellular vesicles released from mesenchymal stromal cells modulate miRNA in renal tubular cells and inhibit ATP depletion injury. *Stem cells and development*. 2014. doi: [10.1089/scd.2013.0618](https://doi.org/10.1089/scd.2013.0618)
40. Bian S, Zhang L, Duan L, Wang X, Min Y, Yu H. Extracellular vesicles derived from human bone marrow mesenchymal stem cells promote angiogenesis in a rat myocardial infarction model. *Journal of molecular medicine*. 2014; 92: 387-397. doi: [10.1007/s00109-013-1110-5](https://doi.org/10.1007/s00109-013-1110-5)
41. Li W, Zhang Q, Wang M, et al. Macrophages are involved in the protective role of human umbilical cord-derived stromal cells in renal ischemia-reperfusion injury. *Stem cell research*. 2013; 10: 405-416. doi: [10.1016/j.scr.2013.01.005](https://doi.org/10.1016/j.scr.2013.01.005)
42. Ratajczak J, Wysoczynski M, Hayek F, Janowska-Wieczorek A, Ratajczak MZ. Membrane-derived microvesicles: important and underappreciated mediators of cell-to-cell communication. *Leukemia*. 2006; 20: 1487-1495.
43. Cocucci E, Racchetti G, Meldolesi J. Shedding microvesicles: artefacts no more. *Trends in cell biology*. 2009; 19: 43-51. doi: [10.1016/j.tcb.2008.11.003](https://doi.org/10.1016/j.tcb.2008.11.003)