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Commentary Open Access

Utilization of Multiphoton Imaging For Real-Time Fate Determination of Mesenchymal Stem Cells in an Immunocompetent Mouse Model

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Abstract

The clinical application of Mesenchymal Stem Cells (MSCs) for the treatment of a variety of diseases is the focus of intense research. Despite large research efforts many questions regarding MSC biology *in vivo* remain unanswered. For instance, we do not know for certain whether MSCs exert their therapeutic effects directly within the target tissue or indirectly by influencing the polarization of other cell types, such as macrophages, which can then home to the target tissue microenvironment. To help address this issue, the application of intravital multiphoton microscopy allows for the determination of the dynamic action of intact MSCs versus endogenous host cells at the target tissue site in real time.

Keywords: Mesenchymal Stem Cell; MSC; Granulocyte; Multiphoton; Intravital; Mouse Model

Short Commentary

Over the past 10 years, the number of publications characterizing Mesenchymal Stem Cells (MSCs) has increased more than 10 fold (Figure 1) [1]. This is due to the realization of MSCs ability to home to sites of disease and tissue injury, as well as to modulate the actions of the immune system [2-16]. The plasticity of these cells and their ability to exhibit either a type 1 or type 2 response [17-19] has made them the focus of intense research for clinical purposes. Even with the increased interest in these cells, debate exists as to the fate of exogenously administered MSCs, including their mode of action and persistence *in vivo* [20,21]. Additional factors that complicate these determinations include the use of mouse [11,14,20] vs. human MSCs (hMSCs) [2,3,13,21], as well as the use of immunodeficient [2,3,12,13,16] vs. immunocompetent [9,15,21] mouse models when studying hMSCs. All of these caveats have led to reports of MSCs persisting *in vivo* for various durations from days [9,20] to weeks [15] after I.V. administration. Additionally, it has been shown that phagocytic granulocytes, composed largely of the monocyte/macrophage lineage, can take up and transport pieces of I.V. administered MSCs in immunocompetent mice [21]. Therefore it is possible that immunomodulatory effects seen upon MSC administration may be secondary to the effects of the MSCs on other host cells, which then home to sites of injury or disease [20]. The use of low resolution imaging modalities, including bioluminescence (BLI), PET and MRI, provides distribution data for administered MSCs in a global sense [8,9,15,22,23]. However, these inherently low-resolution imaging modalities do not address the dynamic intercellular interplay between MSCs and host immune cells, even in studies involving commonly employed immunocompromised mouse models that still contain abundant innate immune cells such as granulocytes which could be involved in the mechanism of action by MSCs *in vivo* [24-27]. Even as the number of MSC studies continues to grow exponentially, high-resolution *in vivo* imaging studies, especially dynamic intravital two-photon imaging studies, which are capable of addressing some of these questions *in vivo*, are lacking (Figure 1). It is therefore particularly important to devise a system to better determine the fate of these cells, their mode of action, and the role of other cells affected by MSCs. In order to address this issue, we've employed a fully immunocompetent mouse model developed by Thomas Graf [28]. In this mouse model, granulocytes are labeled by insertion of the Green Fluorescent Protein (GFP) into the Lysozyme M (LysM) locus. Additionally, it was shown that the differential expression levels of LysM by neutrophils ($LysM^{hi}$) and monocytes ($LysM^{lo}$) allow for the differentiation of these cells by their corresponding GFP expression. The administration of fluorescently labeled MSCs into these mice, in conjunction with high-resolution intravital multiphoton microscopy, now provides a platform for the direct determination of MSC's homing potential to tissues such as the bone marrow. Furthermore, this imaging technique allows the differentiation between intact MSCs and granulocytes that have taken up cellular debris from the injected MSCs (Figure 2) [21]. Long-term tracking (minutes to hours) of the dynamic, individual interactions of these cells with any other cell type can provide cellular data that are far more relevant than *in vitro* tissue culture approaches or static imaging approaches via low-resolution imaging or high-definition histologic examination. For example, advances in the intravital multiphoton approach would allow scientists to determine if the accumulation of MSC signal seen in the tumor microenvironment is due to the direct homing of exogenously applied MSCs or to tissue macrophages which have taken up the MSC debris, and, in the process, may have been polarized functionally by the MSC to adopt a type 1 or type 2 immune response. Lastly, it should be possible to utilize high-resolution intravital microscopy in conjunction with transgenic MSC cell lines expressing fluorescent proteins under the control of tissue-specific promoters, such as MyoD or Osterix for example, to determine the ultimate fate of these cells *in vivo*. These biological insights at the tissue level hold the key in further refining how MSCs can be used more effectively and efficiently in clinical applications.

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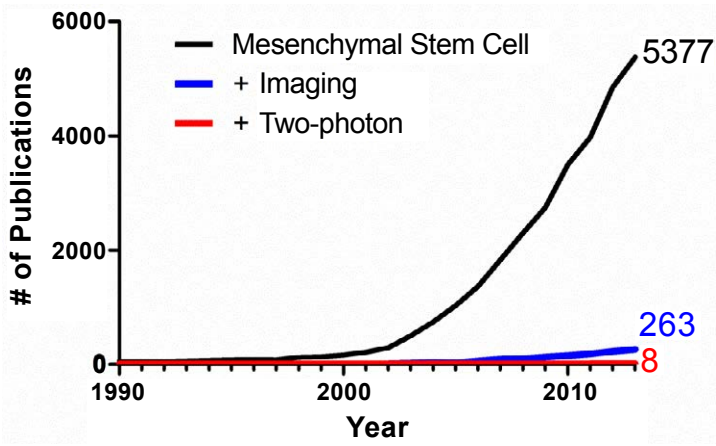

Figure 1: Number of MSC related publications by year. Graph of the number of PubMed publications referencing "mesenchymal stem cell", "mesenchymal stem cell imaging", and "mesenchymal stem cell two-photon" by year from 1990 through 2013. The numbers at the end of each trend line represents the number of publications returned for 2013.

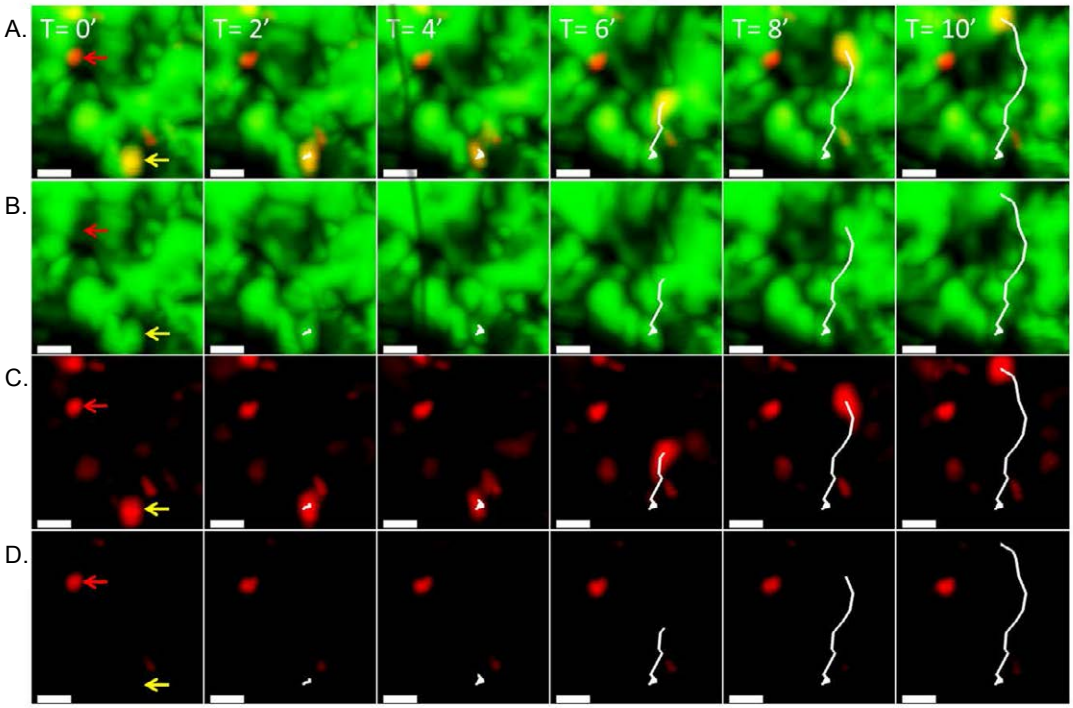

Figure 2: Two-photon imaging of fluorescently labeled MSCs in the bone marrow of a LysM+/GFP mouse. Real-time imaging of Cell Tracker Orange (CTO) labeled hMSCs in the bone marrow of a LysM+/GFP mouse 1 day after I.V. administration allows for the determination of intact MSCs (red arrows) versus MSCs phagocytosed by GFP+ granulocytes (yellow arrows). The paths of individual cells are tracked (white line). A. Combined GFP and CTO channels with representative pictures over a 10-min period. B. GFP-only channel. C. CTO only channel. D. CTO channel after subtraction of phagocytosed CTO signal. Note: Scale bars=20 μm.

References

1. Corlan AD (2004) Medline trend: automated yearly statistics of PubMed results for any query.
2. Beckermann BM, Kallifatidis G, Groth A, Frommhold D, Apel A, et al. (2008) VEGF expression by mesenchymal stem cells contributes to angiogenesis in pancreatic carcinoma. Br J Cancer 99: 622-631. [PubMed]
3. Chu Y, Liu H, Lou G, Zhang Q, Wu C (2014) Human placenta mesenchymal stem cells expressing exogenous kringle1-5 protein by fiber-modified adenovirus suppress angiogenesis. Cancer Gene Ther 21: 200-208. [PubMed]
4. Sage EK, Kolluri KK, McNulty K, Lourenco SD, Kalber TL, et al. (2014) Systemic but not topical TRAIL-expressing mesenchymal stem cells reduce tumour growth in malignant mesothelioma. Thorax 69: 638-647. [PubMed]
5. Choi EW, Shin IS, Park SY, Park JH, Kim JS, et al. (2012) Reversal of serologic, immunologic, and histologic dysfunction in mice with systemic lupus erythematosus by long-term serial adipose tissue-derived mesenchymal stem cell transplantation. Arthritis Rheum 64: 243-253. [PubMed]
6. dos Santos CC, Murthy S, Hu P, Shan Y, Haitsma JJ, et al. (2012) Network analysis of transcriptional responses induced by mesenchymal stem cell treatment of experimental sepsis. Am J Pathol 181: 1681-1692. [PubMed]
7. Hannoush EJ, Elhassan I, Sifri ZC, Mohr AA, Alzate WD, et al. (2013) Role of bone marrow and mesenchymal stem cells in healing after traumatic injury.

Surgery 153: 44-51. [PubMed]

8. Kantarcioglu M, Caliskan B, Demirci H, Karacalioglu O, Kekilli M, et al. (2014) The efficacy of mesenchymal stem cell transplantation in caustic esophagus injury: an experimental study. Stem Cells Int 2014: 939674. [PubMed]

9. Wise AF, Williams TM, Kiewiet MB, Payne NL, Siatskas C, et al. (2014) Human mesenchymal stem cells alter macrophage phenotype and promote regeneration via homing to the kidney following ischemia-reperfusion injury. Am J Physiol Renal Physiol 306: F1222-1235. [PubMed]

10. Krasnodembskaya A, Samarani G, Song Y, Zhuo H, Su X, et al. (2012) Human Mesenchymal Stem Cells Reduce Mortality and Bacteremia in Gram Negative Sepsis in Mice in Part by Enhancing the Phagocytic Activity of Blood Monocytes. Am J Physiol Lung Cell Mol Physiol 302: L1003-1013. [PubMed]

11. Schlosser S, Dennler C, Schweizer R, Eberli D, Stein JV, et al. (2012) Paracrine effects of mesenchymal stem cells enhance vascular regeneration in ischemic murine skin. Microvasc Res 83: 267-275. [PubMed]

12. Dreger T, Watson JT, Akers W, Molligan J, Achilefu S, et al. (2014) Intravenous application of CD271-selected mesenchymal stem cells during fracture healing. J Orthop Trauma 28: S15-19. [PubMed]

13. Bayo J, Fiore E, Aquino JB, Malvicini M, Rizzo M, et al. (2014) Increased Migration of Human Mesenchymal Stromal Cells by Autocrine Motility Factor (AMF) Resulted in Enhanced Recruitment towards Hepatocellular Carcinoma. PLoS One 9: e95171. [PubMed]

14. Nauta AJ, Westerhuis G, Kruisselbrink AB, Lurvink EG, Willemze R, et al. (2006) Donor-derived mesenchymal stem cells are immunogenic in an allogeneic host and stimulate donor graft rejection in a nonmyeloablative setting. Blood 108: 2114-2120. [PubMed]

15. Liu L, Yu Y, Hou Y, Chai J, Duan H, et al. (2014) Human umbilical cord mesenchymal stem cells transplantation promotes cutaneous wound healing of severe burned rats. PLoS One 9: e88348. [PubMed]

16. Leng L, Wang Y, He N, Wang D, Zhao Q, et al. (2014) Molecular imaging for assessment of mesenchymal stem cells mediated breast cancer therapy. Biomaterials 35: 5162-5170. [PubMed]

17. Lei J, Wang Z, Hui D, Yu W, Zhou D, et al. (2011) Ligation of TLR2 and TLR4 on murine bone marrow-derived mesenchymal stem cells triggers differential effects on their immunosuppressive activity. Cell Immunol 271: 147-156. [PubMed]

18. Waterman RS, Tomchuck SL, Henkle SL, Betancourt AM (2010) A new mesenchymal stem cell (MSC) paradigm: polarization into a pro-inflammatory MSC1 or an Immunosuppressive MSC2 phenotype. PLoS One 5: e10088. [PubMed]

19. Delarosa O, Dalemans W, Lombardo E (2012) Toll-like receptors as modulators of mesenchymal stem cells. Front Immunol 3: 182. [PubMed]

20. Eggenhofer E, Benseler V, Kroemer A, Popp FC, Geissler EK, et al. (2012) Mesenchymal stem cells are short-lived and do not migrate beyond the lungs after intravenous infusion. Front Immunol 3: 297. [PubMed]

21. Myers JT, Barkauskas DS, Huang AY (2013) Dynamic Imaging of Marrow-Resident Granulocytes Interacting with Human Mesenchymal Stem Cells upon Systemic Lipopolysaccharide Challenge. Stem Cells Int 2013: 656839. [PubMed]

22. Perez-Galarza J, Carlotti F, Rabelink MJ, Cramer S, Hoeben RC, et al. (2014) Optimizing reporter constructs for *in vivo* bioluminescence imaging of IFN-γ stimulated mesenchymal stromal cells. Exp HematolIn Press. [PubMed]

23. Ribot EJ, Gaudet JM, Chen Y, Gilbert KM, Foster PJ (2014) *In vivo* MR detection of fluorine-labeled human MSC using the bSSFP sequence. Int J Nanomedicine 9: 1731-1739. [PubMed]

24. Di Carlo E, Forni G, Lollini P, Colombo MP, Modesti A, et al. (2001) The intriguing role of polymorphonuclear neutrophils in antitumor reactions. Blood 97: 339-345. [PubMed]

25. Lozupone F, Luciani F, Venditti M, Rivoltini L, Pupa S, et al. (2000) Murine granulocytes control human tumor growth in SCID mice. Int J Cancer 87: 569-573. [PubMed]

26. Morin J, Chimènes A, Boitard C, Berthier R, Boudaly S (2003) Granulocyte-dendritic cell unbalance in the non-obese diabetic mice. Cell Immunol 223: 13-25. [PubMed]

27. Ueda Y, Kondo M, Kelsoe G (2005) Inflammation and the reciprocal production of granulocytes and lymphocytes in bone marrow. J Exp Med 201: 1771-1780. [PubMed]

28. Faust N, Varas F, Kelly LM, Heck S, Graf T (2000) Insertion of enhanced green fluorescent protein into the lysozyme gene creates mice with green fluorescent granulocytes and macrophages. Blood 96: 719-726. [PubMed]