

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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Received June 20, 2014; Accepted July 17, 2014; Published July 19, 2014

Citation: Myers JT, Petrosiute A, Huang AY (2014) Utilization of Multiphoton Imaging For Real-Time Fate Determination of Mesenchymal Stem Cells in an Immunocompetent Mouse Model. J Stem Cell Res Ther 4: 217. doi:10.4172/2157-7633.1000217

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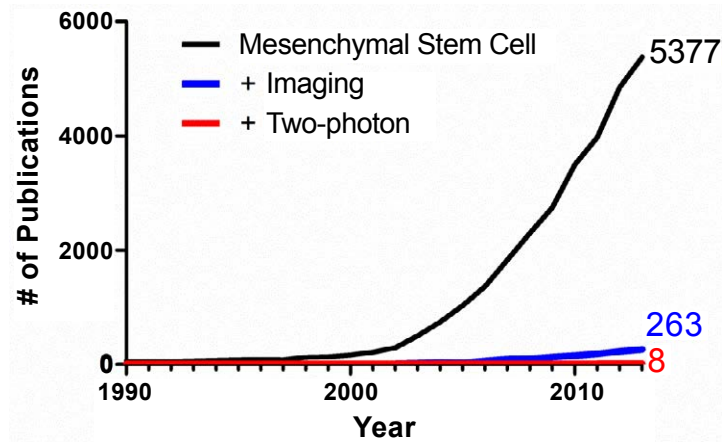


Figure 1: Number of MSC related publications by year. Graph of the number of PubMed publications referencing “mesenchymal stem cell”, “mesenchymal stem cell imaging”, and “mesenchymal stem cell two-photon” by year from 1990 through 2013. The numbers at the end of each trend line represents the number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

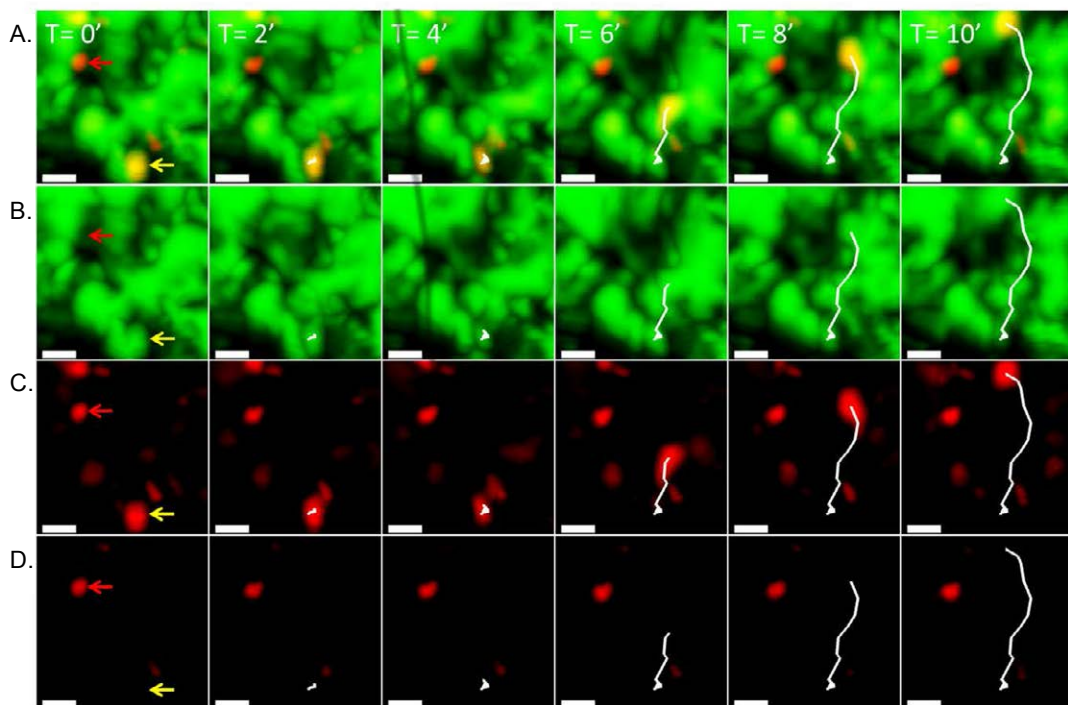


Figure 2: Two-photon imaging of fluorescently labeled MSCs in the bone marrow of a LysM+/GFP mouse. Real-time imaging of Cell Tracker Orange (CTO) labeled hMSCs in the bone marrow of a LysM+/GFP mouse 1 day after I.V. administration allows for the determination of intact MSCs (red arrows) versus MSCs 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 μ m.

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