Hypoxia regulates the pro-angiogenic effect of subcutaneously transplanted mesenchymal stromal cells

Mihai Bogdan Preda, Maya Simionescu, Sinziana Popescu, Raluca Tutuianu, Ana-Maria Rosca, Alexandrina Burlacu

Institute of Cellular Biology and Pathology „Nicolae Simionescu", Bucharest, Romania

Introduction. Cell therapy could be an attractive option in the effort to achieve cardiac regeneration after infarction. Owning to their pro-angiogenic and trophic effects, multipotent mesenchymal stromal cells (MSCs) have become one of the most useful sources in the field of regenerative medicine for myocardial reconstructive therapy. Reports from our and other laboratories have showed that MSCs not only secrete molecules with paracrine protective effects but also exert remote, endocrine-like acting protection, suggesting that homing to the site of injury is not a stringent requirement for achieving therapeutic efficacy. Here we report that subcutaneously (s.c.) transplanted MSCs give rise to multicellular hypoxic aggregates that stimulate angiogenesis at the site of transplantation, and thus evenly contribute to graft survival and to distribution of protective molecules at distant sites.

Comment [S1]: Owning to their pro-angiogenic and trophic effects, multipotent mesenchymal stromal cells (MSCs) have become one of the most attractive sources for cell therapy in the effort to achieve cardiac regeneration after infarction.

Methods and Results. MSCs obtained from bone marrow of male C57BL/6 mice were characterized and used between 4^{th} and 9^{th} passages. S.c. transplantation of CMTPX-labeled cells in syngenic mice in three distinct anatomic regions (interscapular, inguinal and abdominal) followed by IVIS analysis demonstrated that the adjacent adipose tissue did not influence the engraftment and survival of transplanted cells.

To evaluate the correlation between the transplanted cell dose and cell survival within the multicellular aggregate produced in vivo after s.c. transplantation, animals were injected in the interscapular region with a low dose ($1x10^6$ cells) or a high dose ($3.5x10^6$ cells) of luciferase-expressing MSCs, followed by IVIS analysis. A time scale evaluation of the bioluminescence signal of the grafts showed that the number of viable cells was comparable in the two groups at 7 days after transplantation, thus suggesting that cell viability entailed restrictions in the transplantation dose. Moreover, cell survival appeared to be closely related to locally-produced angiogenesis, as large-size aggregates stimulated angiogenesis at a reduced level compared to low-size aggregates.

Since angiogenesis is directly associated to hypoxia-mediated signaling pathways, hypoxia level was quantified in cell aggregates at 2 and 7 days after s.c. transplantation, making use of the fluorescent imaging agent HypoxiSense 680. The results confirmed that high-dose aggregates yielded a significantly increased hypoxia signal, as compared to low-dose aggregates. To further analyze the hypoxia-activated pathways in MSC aggregates, the accumulation of HIF-1a and miR-210, two fundamental regulators of hypoxia, was evaluated by in vivo imaging. Interestingly, while HIF-1α activation steadily decreased within the first 4 days after transplantation, the activation of miR-210 persisted during that period, which might indicate other, yet unknown, functions of miR-210, which extends beyond cell response to hypoxia.

Conclusion. Subcutaneously transplanted MSC form multicellular aggregates that stimulate local angiogenesis. Angiogenesis is directly correlated with the level of hypoxia in the aggregates and with their size. These data sustain the existence of an endocrine mediated protective effects of subcutaneously transplantated MSCs, a process by which the cells can generate protection without the need of homing to the site of injury.

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