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Pro-angiogenic and pro-survival functions of glucose in human mesenchymal stem cells upon transplantation

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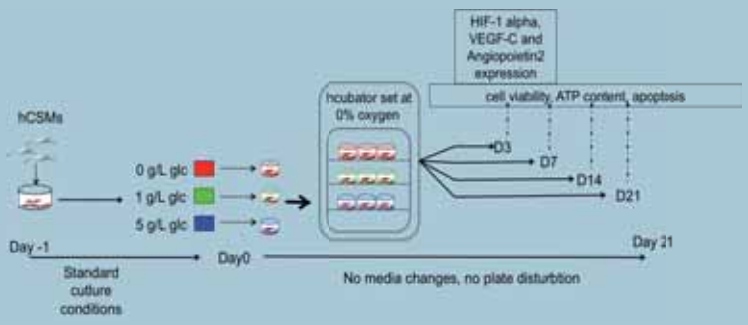
Abstract

The survival of human mesenchymal stem cells (hMSCs) has elicited a great deal of interest, because it is relevant to the efficacy of engineered tissues. In this study, we challenged the current paradigm of hMSC survival, which assigned a pivotal role to oxygen, by testing the hypothesis that exogenous glucose may be key to hMSC survival. We demonstrated that hMSCs could endure sustained near-anoxia under conditions of sufficient glucose. In this cellular model, Hif-1 alpha, a key regulator of cell survival and angiogenesis, was up-regulated by glucose. In addition we demonstrated in vivo that 3D constructs supplemented with glucose implanted into a mouse model exhibited 4–5-fold higher viability and a better peripheral vascularization compared to those without glucose. These findings provided the first direct in vitro and in vivo demonstration that glucose alone significantly reinforces hMSC survival and tissue construct vascularization.

Objectives

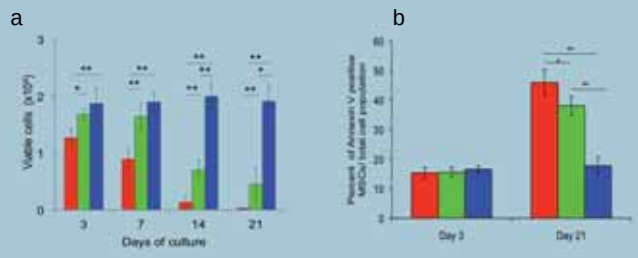
To provide the first direct *in vitro* and *in vivo* demonstration of the pro-angiogenic and pro- survival functions of glucose in hMSC upon transplantation and identified glucose as an essential component of the ideal scaffold for transplanting stem cells.

Methods

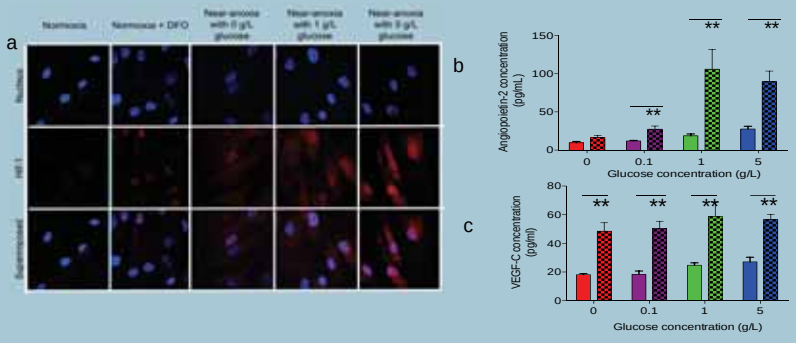


Results (1)

In vitro study



Abrupt or cell-driven ischemia but not sustained near anoxia affected hMSC viability: (a) Cell viability when MSCs were cultured with 0, 1 or 5g/l of glucose(red, green and blue bars respectively); (c) Anexin V positive cells at day 3) or 21 under anoxia when cells were cultured in the presence of various concentration of glucose (p<0.001) (Anova Test)



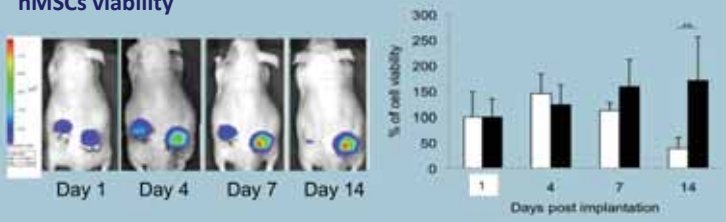
Glucose regulated Hif-1 alpha, Angiopoietin and VEGF-C expression : Confocal imaging (**a**) of Hif-1 alpha Quantification of angiopoietin-2 **(b)** and VEGF- C **(c)** in the supernatant medium of MSCs when hMSCs were cultured under near anoxia with either 0, 1, or 5 g/L glucose at day 3 and day 7 (shaded bar). Data are represented as mean +/- SD. *p<0.05 and **p<0.001

These results demonstrate that glucose depletion is detrimental to MSCs survival under anoxia. Moreover, the presence of glucose enhance HIF-1 alpha expression as well as its bioactivity and the secretion of angiogenic markers such as Angiopoietin and VEGF. Taken together these data suggest that glucose is essential to cell survival and cell response to hypoxia.

Results (2)

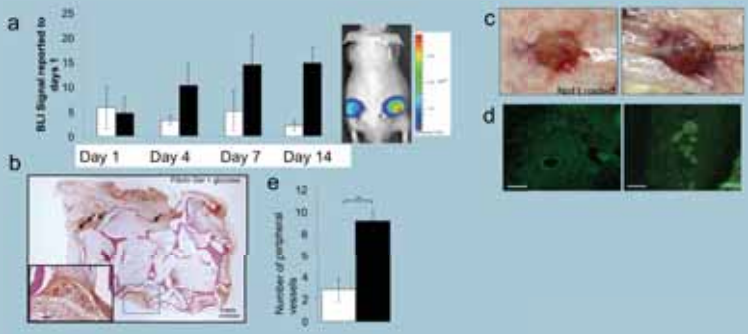
In vivo study

hMSCs viability



Glucose enhances *in vivo* cell survival in a 3D construct.Imaging and quantification of hMSC viability seeded in AN69 scaffolds filled with hyaluronic acid (2%) loaded (black bars/ right side) or not (white bars/left side) with glucose.

Vascularization



Glucose enhanced HIF-1 bioactivity and vascularization of 3D construct: (a) quantification and imaging (at day 14) of HRE expression in hMSC seeded in AN69 scaffolds filled with fibrin gel loaded (black bars) or not (white bars) with glucose. (b) Representative histology results of peripheral vascularization (black arrows) of 3D constructs loaded with glucose after 2 weeks of subcutaneous implantation in mice. Stain: Hematoxylin-Eosine-Safarin; (magnification: x2) including magnification (x10) of region of interest. (c) representative photograph of the 3D construct at day 14 when loaded without (left photograph) or with (right photograph) glucose. (d) immuno-staining of blood vessels by isolectin B4, magnification (x10) (e) Peripheral vascularization quantification in 3D

Results show a striking increase of cell viability (4 fold) when the cell were implanted in the presence of glucose, moreover this *in vivo* study confirms results obtained in vitro demonstrating that glucose supply enhance HIF-1 bioactivity in the implanted hMSCs as well as peripheral implant vascularization.

Conclusions

The present study was the first to provide in vitro and in vivo demonstrations that glucose (but not glutamine) significantly enhanced the ability of hMSCs to survive in a near-anoxic environment. At last but not the least, in vitro and in vivo glucose supply significantly enhances Hif-1 alpha expression and angiogenesis by the secretion of angiogenic factors such as Angiopoietin and VEGF-C. This finding provides valuable insights to current understanding of the mechanisms underlying MSC death upon implantation.