Disponible en ligne sur
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com

TRANSFUSION
CLINIQUE ET BIOLOGIQUE

Transfusion Clinique et Biologique xxx (2017) xxx–xxx

Research perspectives

To harness stem cells by manipulation of energetic metabolism

Manipuler le métabolisme énergétique pour exploiter les cellules souches

D. Loncaric , P. Duchez , Z. Ivanovic *

Inserm, université de Bordeaux, établissement français du sang Aquitaine-Limousin & U1035, 33076 Bordeaux cedex, France

Abstract

The maintenance of the primitive Hematopoietic Stem Cells (HSC) in course of ex vivo expansion is a critical point to preserve the long-term reconstituting capacity of a hematopoietic graft. On the basis of the numerous experimental results, the maintenance of primitive HSC is related to their specific metabolic profile shifted towards the anaerobiosis. Hence, in addition to the exposition of the cultures to more appropriate, physiologically low O_2 concentrations (usually misleadingly termed "hypoxia"), a specter of "hypoxia-mimicking" factors (cytokines, growth factors, receptor-ligands, antioxidants) can be applied to maintain this specific metabolic profile enabling an appropriate genetic and epigenetic regulation. Some factors already proved to be able to achieve this goal and "hypoxia-mimicking" ex vivo cultures were already used to produce cells for clinical trials. In this article we are discussing the approaches aimed to amplify and/or to condition the HSC, based on the manipulation of energetic metabolism properties.

Keywords: Hematopoietic stem cells; Energetic metabolism; Anaerobiosis; Self-renewal; Ex vivo expansion

Résumé

Le maintien des cellules souches hématopoïétiques (CSH) au cours de l'expansion ex vivo est un point critique pour préserver la capacité de reconstitution à long-terme d'un greffon hématopoïétique. En se basant sur les nombreux résultats expérimentaux, le maintien des CSH primitives est lié à leur profil métabolique spécifique orienté vers l'anaérobiose. En conséquence, en plus de l'exposition à des concentrations physiologiques à faible teneur en O_2 plus appropriées (habituellement nommées à tort « hypoxie »), un ensemble de facteurs mimant l'effet d'une basse concentration en O_2 (cytokines, facteurs de croissance, récepteurs ligands, antioxydants) peuvent être appliqués pour obtenir le profil métabolique spécifique permettant une régulation génétique et épigénétique appropriée. Certains facteurs se sont avérés atteindre ce but, et les cultures ex vivo « mimant l'hypoxie » ont déjà été utilisées pour produire des cellules pour des essais cliniques. Dans cet article, nous discutons des approches destinées à amplifier et/ou conditionner les CSH basées sur la manipulation des propriétés du métabolisme énergétique.

Mots clés : Cellules souches hématopoïétiques ; Métabolisme énergétique ; Anaérobiose ; Auto-renouvellement ; Expansion ex vivo

1. Introduction

Important limitations for optimal exploitation of Hematopoietic Stem Cells (HSC) in regenerative medicine are related to their restricted availability, as well as low survival after injection. To improve HSC transplantation efficiency ex vivo manipulation is an attractive approach to obtain sufficient quantity and quality of cells for therapy. However, the most pronounced problem related to ex vivo manipulation of HSC is losing ability of long-term engraftment [1].

Ex vivo cultures of SC are complex systems endowed by physical and chemical properties including partial pressure of oxygen in system, presence and concentrations of different growth factors and cytokines [2]. Efficient systems for ex vivo cultures should be able to gain two opposite results: maintaining

* Corresponding author. Établissement français du sang Aquitaine-Limousin, CS21010, place Amélie-Raba-Léon, 33075 Bordeaux cedex, France.
E-mail address: zoran.ivanovic@efs.sante.fr (Z. Ivanovic).

http://dx.doi.org/10.1016/j.tracli.2017.05.006
1246-7820/

or amplification of HSC as well as generation of committed progenitors [1]. To reach this goal, it is of crucial importance to understand core molecular mechanisms guiding simple cell divisions and self-renewal capacity of HSC, as well as to explain molecular mechanisms inducing a shift to commitment and differentiation.

2. The metabolic profile and function of HSC

The central player in maintaining this fine balance between self-renewal and commitment process is specific energetic metabolic profile of HSC [2]. These cells are characterized by anaerobic glycolytic energetic metabolism, low mitochondrial mass and low Oxidative Phosphorylation activity (OXPHs). Glycolysis is process by which cells produce ATP and reduce NDPH/H [3]. In absence of O_2, this process occurs, but the yield, per one molecule of glucose is low. Through this process they avoid toxic Reactive Oxygen Species (ROS) generation, in the same time providing precursors for pentose phosphate pathway and inducing glutton oxidase regeneration. Energy produced by anaerobic metabolic way is sufficient to support slow cell divisions not associated with commitment and differentiation, which is, in fact, self-renewal [4]. Shift to OXPHs metabolism, higher mitochondrial mass and activity are observed during the process of HSC lineage commitment and further differentiation. That shift to aerobic metabolism is significant since providing a much higher yield of ATP production per one molecule of glucose comparing to anaerobically occurring glycolysis. That's why energy produced by OXPHs can be sufficient to support cells in course of differentiation characterized by activation of specialized functions [5]. Key significance of this fine metabolic balance between glycolysis and OXPHs for regulating self-renewal and differentiation ratio is confirmed by observing reverse events during reprogramming of terminally differentiated somatic cells to produce induced Pluripotent Stem Cells (iPS) [6].

3. Ex vivo culture oxygenation as the key factor

As it is already stressed out, the partial pressure of oxygen for ex vivo culturing systems is of paramount importance. Unfortunately, atmospheric oxygen concentration is still accepted as standard culture condition, which is highly hyperoxic state comparing to real oxygen concentration in SC microenvironment, which is very low [7].

Experiments performed at physiological relevant oxygen concentrations (1–5% O_2), prove better ex vivo maintenance of SCs and their proliferative potential, comparing to those performed at atmospheric oxygen concertation (20% O_2), condition, mainly considered as "normoxia" representing, in fact, the real hyperoxia [2].

Culturing of HSC in physiological relevant oxygen concentrations improves ex vivo maintenance of HSC and enables their proliferation. All details concerning molecular mechanisms by which low oxygen concentration impacts SC features are not yet completely revealed. Still, the main mechanisms of low oxygen concentration action seem to be clear: (1) attenuating of mitochondrial respiratory chain activity, (2) inducing a shift towards and maintaining glycolytic energetic metabolism, (3) impacting ROS levels and related signaling cascades as well as (4) stabilizing of Hypoxia Induced Factor (HIF) proteins family [1].

4. Influencing the functional HSC properties by controlling their energetic metabolism through modulation of "hypoxic" signaling

Presence and concentrations of different cytokines/growth factors, antioxidants and other substances acting as hypoxia-mimicking agents in cultures could be also beneficial, to maintain proper quality and quantity of cells for therapy [8].

Thrombopoietin (TPO), well established as primary regulator of platelet formation, could induce HIF-1 α stabilization in a manner not related to O_2 concentration, inducing hypoxia-like physiological response of HSC and attenuating effects of high, atmospheric oxygen concentration [9]. Applied by itself TPO can improve ex vivo culture conditions, but it can also act in synergy with relevant oxygen concentrations or/and other hypoxia mimicking factors [10]. Exact molecular mechanism by which HIF-1 α is stabilized in TPO-dependent manner is not completely understood. There are indications that ROS-mediated signaling is crucial for this mechanism, suppressing activity of Prolyl Hydroxylase (PHD), which is necessary to label HIF-1 α for ubiquitination. HIF-1 α as transcription regulator is important to support glycolytic metabolic profile, low mitochondrial activity and genes regulation involved in maintaining a balance of stem cell properties and differentiation [9].

Stem Cell Factor (SCF), a constitutive component of SC niche, is also reported to have similar effects on HIF-1α stabilization, but it is also related to stabilization of HIF-2 [10]. The mechanisms by which SCF is involved in HIF-1 and HIF-2 stabilization are different than those mediated by TPO. SCF increases level of HIF-1 and HIF-2 gene transcripts and supports stabilization of protein, a molecular mechanism that is not well understood [11]. It is established, however, that the action of SCF is mediated by activation of RAS/MEK/ERK and PI3 K/A [10].

It is reported that a cocktail of cytokines containing TPO and SCF in combination with an appropriate medium, significantly improves survival and proliferation of HSC and Hematopoietic committed Progenitor Cells (HPC) in ex vivo cultures. These cultures are developed to the clinical grade and exploited for clinical trials [12].

Notch signaling is important part of cellular response to hypoxia [1]. An intracellular domain of activated Notch1 receptor (ICD) interacts directly with HIF-1α protein and, in non-canonical way, independent of HIF-1β, regulates gene expression related to hypoxia. Also, a cross talk was described between protein stabilization of HIF- 1α and ICD of Notch [13].

Thus, Notch signaling manipulation and Notch ligands application could be of a great interest to improve ex vivo amplification of HSC, which is proved in experimental ways and clinical trials.

Please cite this article in press as: Loncaric D, et al. To harness stem cells by manipulation of energetic metabolism. Transfusion Clinique et Biologique (2017), http://dx.doi.org/10.1016/j.tracli.2017.05.006