Molecular Aspects of Medicine 47–48 (2016) 15–23

Contents lists available at ScienceDirect

Molecular Aspects of Medicine

journal homepage: www.elsevier.com/locate/mam

Review

Dynamic regulation of stem cell specification and maintenance by hypoxia-inducible factors

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ARTICLE INFO

Article history:
Received 28 August 2015
Accepted 8 September 2015
Available online 5 November 2015

Keywords:
Hematopoietic stem cells
Embryonic stem cells
Induced pluripotent stem cells
Glioma stem cells
Leukemia stem cells
Breast cancer stem cells

ABSTRACT

Stem cells are characterized by the capacity for both self-renewal and generation of all other cell types (pluripotency) or differentiated cells within a particular lineage (multipotency). Stem cells are often localized to hypoxic niches within tissues and hypoxia inducible factors (HIFs) play key roles in the maintenance of pluripotent and multipotent stem cells, as well as cancer stem cells, which are also known as tumor-initiating cells. HIF inhibitors target cancer stem cells and improve the responses to angiogenesis inhibitors and cytotoxic chemotherapy in mouse models of breast cancer.

Contents

1. Introduction

Oxygen is essential for multicellular life because of its critical role in respiration, which provides sufficient energy to construct and maintain complex life forms. Among

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http://dx.doi.org/10.1016/j.mam.2015.09.004
0098-2997/

metazoan species, hypoxia-inducible factors (HIFs) serve as master regulators of oxygen homeostasis by balancing O_2 delivery and utilization. This balance is required for survival, because too little or too much O_2 leads to cell death as a result of energy depletion and/or reactive oxygen species (ROS) toxicity. As a result, O_2 availability modulates many biological processes, including the specification and maintenance of stem cells.

HIF-1 is composed of HIF-1α and HIF-1β subunits (Wang and Semenza, 1995; Wang et al., 1995) and is found in all metazoan species (Loenarz et al., 2011). HIF-1α is subject to O_2-dependent hydroxylation, VHL-dependent ubiquitination, and proteasomal degradation, such that HIF-1α protein levels increase exponentially as O_2 levels decrease (reviewed in Prabhakar and Semenza, 2012). HIF-1 binds to the DNA sequence 5'-(A/G)CGTG-3', recruits coactivators, and activates the transcription of genes located in cis. The HIF-2α and HIF-3α subunits, which were identified based on their sequence similarity to HIF-1α and are only found in vertebrate species, are also O_2-regulated and dimerize with HIF-1β to activate transcription of target genes (Tian et al., 1997; Zhang et al., 2014), although alternative splicing of the HIF-3α primary RNA generates an isoform, designated iPAS, that binds to HIF-1α and inhibits its activity (Makino et al., 2002). This review will focus on the roles of HIF-1α and HIF-2α in stem cell biology.

2. HIFs in HSCs, ESCs, and iPSCs

HIFs play important roles in the specification and/or maintenance of multipotent and pluripotent stem cells. Hematopoietic stem cells (HSCs), embryonic stem cells (HSCs), and induced pluripotent stem cells (iPSCs) will be discussed here, but hypoxia regulates many other types of stem cells, including kidney stem cells (Oliver et al., 2004), mesenchymal stem cells (Fehrer et al., 2007), muscle stem cells (Gustafsson et al., 2005), and neural stem cells (Mohyeldin et al., 2010).

2.1. HSCs

HSCs are the most well studied stem cells and they have served as a paradigm for stem cell biology (Orkin and Zon, 2008). As is the case for all stem cells, they are defined by the properties of self-renewal and multipotency. HSCs undergo asymmetric cell divisions that give rise to daughter HSCs (self-renewal) as well as to progenitor cells that proliferate and differentiate to give rise to all of the cell types in the hematopoietic lineage (multipotency). This involves a balance because increasing the number of daughter progenitor cells, relative to daughter HSCs, will allow for rapid increases in hematopoietic cells but if continued will eventually result in HSC depletion. O_2 concentrations in bone marrow range from ~6% (42 mm Hg) near the blood vessels (vascular niche) to ~1% (7 mm Hg) near the bone (endosteal niche) (Eliasson and Jonsson, 2010; Kiel and Morrison, 2008).

Several lines of experimental evidence indicate that hypoxia tips the balance toward the HSC phenotype. First, compared to standard tissue culture conditions (20% O_2), ex vivo culture of human bone marrow cells under hypoxic conditions (1.5% O_2) leads to an expansion of the HSC population (Danet et al., 2003). Second, HSCs are preferentially localized within hypoxic regions of the mouse bone marrow (Parmar et al., 2007). Third, targeted deletion of the *Hif1a* gene encoding HIF-1α in mouse hematopoietic cells results in failure of lineage reconstitution after serial bone marrow transplantation due to HSC depletion (Takubo et al., 2010). Fourth, conditional *Hif1a* knockout by Cre expression from the promoter of the *Cdh5* gene, which encodes vascular endothelial cadherin, results in reduced numbers of HSCs in mouse embryos (Imanirad et al., 2014). In contrast, loss of HIF-2α expression in HSCs does not affect serial transplantation (Guitart et al., 2013).

Taken together, the data suggest that HIF-1α is required for the maintenance of quiescent, long-term HSCs, which occupy the hypoxic endosteal niche within the bone marrow and are critical for HSC self-renewal. In contrast, cycling, short-term HSCs that give rise to multipotent progenitors and their lineage-restricted progeny, localize to the more highly oxygenated vascular niche (Perry and Li, 2007; Suda et al., 2011). These findings are consistent with a model in which there are either two HSC populations, which remain histologically segregated, or a model with a single, dynamic population of HSCs, which migrate from one niche to the other. HIF-1α has been shown to inhibit DNA replication by both transcriptional (Koshiji et al., 2004) and non-transcriptional (Hubbi et al., 2013, 2014) mechanisms in cancer cell lines, but it is not clear whether these functions are required to maintain the quiescence of long-term HSCs in the hypoxic endosteal niche.

Perhaps one of the most important functions of HIF-1 in HSCs is as a regulator of metabolism (Suda et al., 2011). HIF-1 controls the switch from oxidative to glycolytic metabolism (Fig. 1), which is required for the survival of hypoxic cells because of excessive ROS production by the mitochondrial electron transport chain under hypoxic conditions (Semenza, 2013). In particular, HIF-1 activates the expression of genes encoding pyruvate dehydrogenase kinase (PDK), which blocks the conversion of pyruvate to acetyl CoA for entry into the tricarboxylic acid cycle (Kim et al., 2006; Papandreou et al., 2006). Long-term HSCs are highly glycolytic with low ROS levels (Jang and Sharkis, 2007) and knockout of HIF-1α reduced the expression of PDK2 and PDK4 mRNA and increased mitochondrial ROS levels (Takubo et al., 2013). HSCs from *Pdk2*$^{-/-}$;*Pdk4*$^{-/-}$ mice phenocopied the impaired HSC quiescence and reduced transplantation capacity of *Hif1a*$^{-/-}$ HSCs (Takubo et al., 2013). These data indicate that the metabolic switch mediated by HIF-1-dependent PDK expression is required for maintenance of the long-term HSC phenotype.

2.2. ESCs

Whereas HSCs are multipotent and give rise to all of the hematopoietic cell lineages, ESCs are pluripotent and give rise to cell types of all three germ layers (endoderm, mesoderm, and ectoderm). The transcription factors NANOG, OCT4, and SOX2 collaborate to form a regulatory circuit that is required for the maintenance of undifferentiated human (Boyer et al., 2005) and mouse (Loh et al., 2006) ESCs in culture. Compared to standard culture conditions (95% air/