Molecular Immunology 68 (2015) 527–535

Contents lists available at ScienceDirect

Molecular Immunology

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

Hypoxia-inducible factors regulate T cell metabolism and function

Anthony T. Phan, Ananda W. Goldrath*

Division of Biological Sciences, Molecular Biology Section, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093, USA

ARTICLE INFO

Article history:
Received 2 June 2015
Received in revised form 15 July 2015
Accepted 5 August 2015
Available online 19 August 2015

Keywords:
Metabolism
T cell
Hypoxia-inducible factor
Differentiation
Immunity

ABSTRACT

Resolution of infection requires the coordinated response of heterogeneous cell types to a range of physiological and pathological signals to regulate their proliferation, migration, differentiation, and effector functions. One mechanism by which immune cells integrate these signals is through modulating metabolic activity. A well-studied regulator of cellular metabolism is the hypoxia-inducible factor (HIF) family, the highly conserved central regulators of adaptation to limiting oxygen tension. HIF's regulation of cellular metabolism and a variety of effector, signaling, and trafficking molecules has made these transcription factors a recent topic of interest in T cell biology. Low oxygen availability, or hypoxia, increases expression and stabilization of HIF in immune cells, activating molecular programs both unique and common among cell types, including glycolytic metabolism. Notably, numerous oxygen-independent signals, many of which are active in T cells, also result in enhanced HIF activity. Here, we discuss both oxygen-dependent and -independent regulation of HIF activity in T cells and the resulting impacts on metabolism, differentiation, function, and immunity.

1. Introduction

Protective immunity relies on coordinate responses from numerous cell types in order for host tissues to resist incursion by foreign agents. Infection of tissues by pathogens often drives vast changes in the tissue microenvironment as well as the upregulation of systemic signals necessary for marshalling immune cells to the fight. Recently, it has become appreciated that antigen, cytokines, chemokines, as well as the balance of nutrients and oxygen can play a role in modulating immune cell metabolism thereby affecting cellular fate and function (Pearce et al., 2013; Chang et al., 2014). Further, T cells must traffic from secondary lymphoid tissues to infected sites, experiencing dramatic shifts in microenvironmental signals unique to tissue milieu, which may play a crucial role in influencing T cell metabolism, altering cellular function and cell fate decisions. How such microenvironmental signals are integrated at the transcriptional level is a topic of considerable importance in understanding T cell immunity.

1.1. T cell metabolism—a modulator of T cell responses

It has been noted for decades that the massive proliferation that occurs following lymphocyte activation is accompanied by marked shifts in cellular metabolism (Wang et al., 1976). Quiescent naive T cells primarily rely on oxidative phosophorylation for their energetic demands, but upon activation by cognate peptide and costimulation from antigen presenting cells, T cells rapidly shift towards a reliance on glycolytic metabolism (Pearce et al., 2013; Chang et al., 2014). Glycolytic supply of energy proves to be critical for differentiation and effector function of activated T cells. Following clearance of the pathogen, most effector T cells die, however a small proportion survive and differentiate into long-lived memory populations that exhibit a coordinated return to a reliance on oxidative phosphorylation (Pearce et al., 2013; Chang et al., 2014). Recent work has demonstrated that these metabolic transitions accompany key cell-fate decisions impacting differentiation and effector function of T cell subsets (Michalek et al., 2011; Shi et al., 2011; van der Windt et al., 2012, 2013; Kidani et al., 2013; Chang et al., 2013; Sukumar et al., 2013; O'Sullivan et al., 2014; Yin et al., 2015; Cui et al., 2015; Mascanfroni et al., 2015). Most intriguingly, data from some of these studies argue that differentiation of memory as well as function of effector cells are dependent on utilization of specific metabolic pathways (van der Windt et al., 2012, 2013; Chang et al., 2013; Sukumar et al., 2013; O'Sullivan et al., 2014; Gubser et al., 2013). For example, the transition from predominantly glycolytic metabolism to oxidative phosphorylation, fatty

* Corresponding author. Fax: +1 8585340980.
E-mail addresses: atphan@ucsd.edu (A.T. Phan), agoldrath@ucsd.edu (A.W. Goldrath).

http://dx.doi.org/10.1016/j.molimm.2015.08.004
0161-5890/

acid metabolism, and generation of spare respiratory capacity by antigen-specific $CD8^+$ T cells may be essential for the formation of long-lived functional memory $CD8^+$ T cells (van der Windt et al., 2012). These data have been recently summarized and are beyond the scope of this review (Pearce et al., 2013; Chang et al., 2014). However, understanding molecular regulators of T cell metabolism has emerged as a topic of intense study, and here we will discuss the master regulators of oxygen homeostasis, the Hypoxia-inducible factor transcription factors (HIFs), and their role in regulating T cell metabolism and function.

1.2. HIF—a regulator of cellular metabolism

The importance of cellular metabolism in altering T cell differentiation and function has propelled research into the role of well-established metabolic regulators such as c-Myc, PI3K/AKT, mTOR, Foxo1, and the HIFs in controlling T cell function. HIFs are a family of transcription factors consisting of three alpha subunits, HIF-1α, HIF-2α, and HIF-3α, with differing tissue-specific expression patterns that when stabilized can heterodimerize with HIF-1β, also known as the aryl hydrocarbon receptor nuclear translocator (ARNT), and in the case of HIF-1α and HIF-2α, bind hypoxia response element (HRE) sequences and activate a transcriptional program dedicated to mediating the adaptation of cells to reduced oxygen availability (Bracken et al., 2003; Schwartz et al., 2011; Palazon et al., 2014). HIF-3α's lack of a c-terminal activation domain suggests that it is unable to activate transcription despite being capable of heterodimerizing with HIF-1β and binding HRE sequences. It is currently unclear what role HIF-3α plays in modulating cellular responses to hypoxia (Makino et al., 2002; Maynard et al., 2003). HIFs promote the adaptation of cells to hypoxia primarily by lowering oxygen consumption through the increased expression of two critical glycolytic enzymes, lactate dehydrogenase A (LDHa), which increases the capacity to regenerate NAD+ following reduction of pyruvate produced by glycolysis, and pyruvate dehydrogenase kinase 1 (PDK1), which actively prevents pyruvate from shunting into the TCA cycle (Firth et al., 1994; Kim et al., 2006). Altering these two metabolic checkpoints along with increased expression of other glycolytic enzymes dramatically shifts cellular metabolism away from oxygen-consuming pathways and drives a reliance on glycolysis for the generation of ATP.

Emerging literature has demonstrated a critical role for HIFs in modulating T cell metabolism *via* both oxygen-dependent and oxygen-independent pathways and highlighted its regulation of other novel gene targets influencing effector T cell function and differentiation (Shi et al., 2011; Dang et al., 2011; Finlay et al., 2012; Clambey et al., 2012; Doedens et al., 2013; Mascanfroni et al., 2015). This review will first examine many of the regulators of HIF in T cells, and then what is known about HIF's impact on T cell metabolism, differentiation, and function. We will conclude by addressing outstanding questions regarding HIF's role in T cell biology that present attractive areas of future study.

2. Regulators of HIF activity in T cells

Interest in HIF-regulated transcription in T cells stems from HIF's function as a modulator of metabolism in response to changes in oxygen tension; however, studies in T cells and other immune cell types have revealed that while a primary function of HIFs is the detection and response to low oxygen tensions, a number of other mechanisms regulate HIF activity in an oxygen-independent fashion, allowing T cells to co-opt the HIF pathway for regulation of cellular metabolism and other known transcriptional targets in response to immune stimuli independent of oxygen availability. Here we will cover many of the known regulators of HIF activity in T cells as well as some that remain unexplored in T cell biology, but likely play a role in T cell function and fate given their described function in other cell types.

2.1. Oxygen

As in other cell types, the HIF pathway serves as the central sensor of oxygen tension in T cells and is regulated in an oxygen-dependent fashion (Palazon et al., 2014; McNamee et al., 2013). When T cells are in oxygen-sufficient environments HIFα subunits are rapidly degraded by the proteasome following hydroxylation by prolyl hydroxylase domain proteins (PHDs), primarily PHD2, and ubiquitination by the von Hippel-Lindau tumor suppressor protein complex (pVHL), an E3 ubiquitin ligase that specifically targets hydroxylated HIFα subunits (Fig. 1) (Ivan et al., 2001; Jaakkola et al., 2001). Conversely, when cells enter hypoxic environments, (~1% O_2), PHDs, which require oxygen (O_2) as a cofactor for their function, along with iron(II) and α-ketoglutarate, are unable to hydroxylate HIF on the appropriate proline residues resulting in a loss of pVHL-dependent ubiquitination and subsequent stabilization of HIFα subunits (Ivan et al., 2001; Jaakkola et al., 2001). HIFα can then dimerize with HIF-1β upon translocation into the nucleus and activate HIF target genes. While canonical oxygen-dependent regulation of the HIF pathway remains integral to regulating HIFα stability, interestingly, activation by cognate antigen of T cells appears to "unlock" the oxygen-dependent responsiveness of T cells amplifying stabilization of HIFα subunits as naive cells cultured in 1% O_2 exhibit mild stabilization of HIFα subunits in comparison to those activated and cultured in 1% O_2 (unpublished observation A. Phan and A. Goldrath and (Wang et al., 2014)).

2.2. Prolyl hydroxylase domain proteins and factor inhibiting HIF-1

Hydroxylation of HIFα subunits is the canonical mechanism for regulating HIF activity (Semenza, 2014). Therefore regulation of PHD expression and activity is critical for regulating HIF function. Three PHD isoforms are present in mice and examination of PHD function shows that PHD2 is the default regulator of HIFα stability in most cell types (Appelhoff et al., 2004). *Egln1* (PHD2) is constitutively expressed in T cells and hydroxylates HIFα subunits for subsequent degradation at the steady state. Interestingly, a conserved HRE has been identified and shown in mouse embryonic fibroblasts to drive increased PHD2 expression following extended exposure of cells to hypoxia, suggesting a self-regulating circuit designed to prepare cells for re-entry into oxygen rich environments following hypoxia (Marxsen et al., 2004; Metzen et al., 2005). *Egln3*, which encodes PHD3, is dynamically expressed in T cells following activation suggesting an importance for hydroxylase activity during T cell responses (Heng et al., 2008). PHD3 also appears to be directly regulated by a HRE in a HIF-dependent fashion in *in vitro* experiments in human cancer cell lines (Marxsen et al., 2004; Pescador et al., 2005). Further exploration of PHD expression and activity in the context of T cell activation will be informative for defining regulators of HIF activity in the immune response.

In addition to PHDs, another hydroxylase, the factor inhibiting HIF-1 (FIH), hydroxylates an asparagine residue in the c-terminal activation domain of both HIF-1α and HIF-2α subunits in normoxia (Mahon et al., 2001; Lando et al., 2002). Asparaginyl-hydroxylation blocks the ability of HIFs to bind transcriptional coactivators CREB-binding protein and p300 (Mahon et al., 2001; Lando et al., 2002). This prevents HIF-mediated transcription, providing an additional layer of post-translational regulation of HIFs that escape degradation by the proteasome.