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Tryptophan metabolism and non-hypoxic induction of hypoxia-inducible factor (HIF)

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Abstract. We studied the response of macrophages to activation of Hypoxia inducible factor 1 pathway triggered by hypoxia or, under normoxic conditions by Picolinic, a metabolite of tryptophan. We analyzed the expression of Glutamine: fructose-6-phosphate amidotransferase (GFAT), the rate-limiting enzyme in the hexosamine biosynthetic pathway controlling protein glycosylation. We obtained the first evidence that the GFAT mRNA and protein are constitutively present in mouse macrophages and we demonstrated that the expression is inducible by hypoxia and by the hypoxia-related stimuli picolinic acid (PA). The promoter of GFAT contains the consensus sequence of the Hypoxia responsive element (HRE) in position −74/−65 and we studied the role of HRE on the activation of the promoter by transfecting the macrophage cell lines with appropriate expression vectors containing fragments of the GFAT promoter. We found that GFAT HRE is essential for the transcriptional activation by hypoxia or PA and that HIF1α can augment this response activate GFAT expression. Moreover, we demonstrated that PA is a potent inducer of HIF 1 and HIF2. Comparison of the gene expression profile induced by hypoxia or PA revealed that only a small number of genes are induced by both stimuli like GFAT, despite the activation of HIF-dependent pathways by both stimuli. © 2007 Published by Elsevier B.V.

Keywords: Glutamine; Fructose-6-phosphate amidotransferase; Macrophages; Hypoxia; Picolinic acid

1. Introduction

Glutamine:fructose-6-phosphate amidotransferase (GFAT) is the rate-limiting enzyme in the hexosamine biosynthetic pathway. In the first step of the pathway, this enzyme converts fructose-6-phosphate to glucosamine-6-phosphate, using glutamine as the nitrogen donor. Flux through the hexosamine pathway has been implicated in some of the adverse

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consequences of high glucose; it may play a role in the development of diabetic kidney disease [1–3] and in several cellular responses.

The transcriptional activation of GFAT can control the levels of the enzyme in the cell. GFAT promoter is activated by epidermal growth factor (EGF), and by angiotensin II in rat mesangial cells [4]. However, GFAT is expressed in a wide variety of cell/tissue types [2,5–8], and it is likely its expression is regulated by different stimuli depending on the cell type. We studied the expression of GFAT gene in macrophages that, so far, has not been addressed.

Macrophages (MΦ) are ubiquitous cells which markedly accumulate in areas of inflammation and tumor growth, and their functions are modulated by stimuli that arise from the pathological microenvironment. A common denominator of many pathological processes is represented by low oxygen tension (hypoxia). Hypoxia occurs in cardiovascular, hematological and pulmonary disorders, inflammatory processes, and fibrosis [9]. Areas of low oxygen concentrations are present in solid tumors and are known to contribute to tumor growth, metastatization, and resistance to radio and chemiotherapy [10]. It is now well recognized that hypoxia is an important environmental stimulus capable of modulating the expression of specific genes including those involved in energy metabolism erythropoietin, angiogenesis, vasomotor control, and iron metabolism [9,11–14]. Induction of gene expression by hypoxia is mediated mainly by the hypoxia-inducible factor-1 and 2 (HIF-1,-2), which bind to and transactivate the hypoxia responsive element (HRE) present in the promoter or enhancer elements of many hypoxia-responsive genes [9]. Signals other than hypoxia can activate HIF-1α and induce gene expression through HRE transactivation in normal oxygen conditions [9,11,15,16], including iron chelators such as picolinic acid (PA).

PA is a catabolite of tryptophan which chelates divalent cations, that is found in vivo in the serum of normal donors and its levels are increased by liver degenerative diseases and inflammation [17]. PA is endowed with several biological activities among which the macrophages co-stimulatory property has been extensively characterized [18]. PA shares with hypoxia the ability to act synergistically with IFNγ in inducing the nitric oxide synthase gene expression [19]. We have addressed the question of the impact of HIFα induction on gene transcription by studying the response of murine macrophages to hypoxia or PA.

2. Material and methods

Mouse MΦ cell line ANA-1 was established by infecting fresh bone marrow-derived cells from C57BL/6 mice with the J2 (*v-raf/v-myc*) recombinant retrovirus [20,21]. Cells were maintained at 37 °C in a humidified incubator containing 20% O_2, 5% CO_2 and 75% N_2 referred to as normoxic conditions. Hypoxic conditions (i.e. 1% O_2) were achieved by incubating the cells at 37 °C in a humidified, anaerobic work station incubator (BUG BOX, Ruskinn, UK) flushed for 20 min at a dynamic pressure of 35 psi and a flow rate of 25 L/min with a mixture of 1% O_2, 5% CO_2 and 94% N_2 and then sealed at a positive pressure to reduce atmospheric leaks.

The p-GFAT plasmid contains the full-length of GFAT promoter (corresponding to −1822/+88 relative to the transcription initiation site) was inserted into pGL-2 [22].

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