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Reciprocal role of SIRT6 and Hexokinase 2 in the regulation of autophagy driven monocyte differentiation

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ABSTRACT

Emerging evidences suggest the impact of autophagy on differentiation but the underlying molecular links between metabolic restructuring and autophagy during monocyte differentiation remain elusive. An increase in PPARγ, HK2 and SIRT6 expression was observed upon PMA induced monocyte differentiation. While PPARγ positively regulated HK2 and SIRT6 expression, the latter served as a negative regulator of HK2. Changes in expression of these metabolic modelers were accompanied by decreased glucose uptake and increase in Chibby, a potent antagonist of β-catenin/Wnt pathway. Knockdown of Chibby abrogated PMA induced differentiation. While inhibition of HK2 either by Lonidamine or siRNA further elevated PMA induced Chibby, mitochondrial ROS, TIGAR and LC3II levels; siRNA mediated knock-down of SIRT6 exhibited contradictory effects as compared to HK2. Notably, inhibition of autophagy increased HK2, diminished Chibby level and CD33 expression. In addition, PMA induced expression of cytoskeletal architectural proteins, CXCR4, phagocytosis, acquisition of macrophage phenotypes and release of pro-inflammatory mediators was found to be HK2 dependent. Collectively, our findings highlight the previously unknown reciprocal influence of SIRT6 and HK2 in regulating autophagy driven monocyte differentiation.

1. Introduction

Monocytes are the central players of the innate immune system, and during inflammation monocytes migrate into the tissues where they differentiate into heterogeneous cell populations like macrophages or dendritic cells [1]. Monocyte differentiation is a vital phenomenon as monocytes are programmed to undergo apoptosis in the absence of any kind of differentiating signals [2]. Interestingly, metabolic changes play a crucial role in driving monocyte differentiation [3], and dynamic changes in mitochondrial energy metabolism occur during differentiation [4]. Metabolic profiles also contributes to differing functions of the classically activated type 1 (M1) and the alternatively activated type 2 (M2) macrophages [5] with glycolytic pathway affecting acquisition of specific macrophage phenotypes [6]. SIRT6-a mammalian NAD+-dependent histone deacetylase acts as regulator of glycolytic genes [7], and SIRT6 inhibition blocks monocyte differentiation into dendritic cells [8]. Besides, SIRT6 is a negative regulator of Hexokinase 2(HK2) [9], and the latter regulates cell survival by preventing mitochondrial death pathway and enhancing autophagy [10].

Autophagy is recognized for its pivotal role in macrophage development and differentiation [11]. Metabolic pathways of glycolysis and oxidative phosphorylation are linked to redox homeostasis, and autophagy interfaces with these metabolic pathways to affect mitochondrial function [12]. Intracellular Reactive Oxygen Species (ROS) play a key role in cell differentiation, and mitochondria are an important source of ROS in this process [13,14]. The metabolic modeler TIGAR (TP53-Induced Glycolysis and Apoptosis Regulator) exhibit ROS-dependent-consequences on apoptosis and autophagy [15]. Interestingly, formation of TIGAR-HK2 complex at the mitochondria modulates HK2 activity and controls mitochondrial ROS under hypoxia [16]. Also, we have highlighted a previously unknown function of HK2 in regulating the metabolic and immune component of glioma cells [17]. In addition to its glycolytic role, other non-canonical functions attributed to HK2 includes its ability to affect cytoskeletal architecture [17]. Moreover the involvement of actin cytoskeleton in autophagosome formation has also been reported [18,19]. Although the importance of autophagic pathways for developmental processes is well established, the mechanisms linking metabolic adaptation to autophagic processes in the perspective of monocyte differentiation and function are wanting. Therefore, in the present study we explored the involvement of hexokinase in regulating autophagy driven monocyte differentiation.

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Table 1
Real time PCR Primers.

Target gene	Forward Primer	Reverse Primer
MCP-1	5′-ACTCTCGCCTCCAGCATGAA-3′	5′-TGATTGCATCTGGCTGAGC-3′
Mannose Receptor	5′-GCCAAATGACGAATTGTGGA-3′	5′-CACGAAGCCATTTGGTAAACG-3′
NOS2	5′-GTTCTCAAGGCACAGGTCTC-3′	5′-GCAGGTCACTTATGTCACTTATC-3′
Arginase-1	5′-GTGGACAGACTAGGAATTGGC-3′	5′-TCCAGTCCGTCAACATCAAAAC-3′
18S	5′-CAGCCACCCGAGATTGAGCA-3′	5′-TAGTAGCGACGGGCGGTGTG-3′

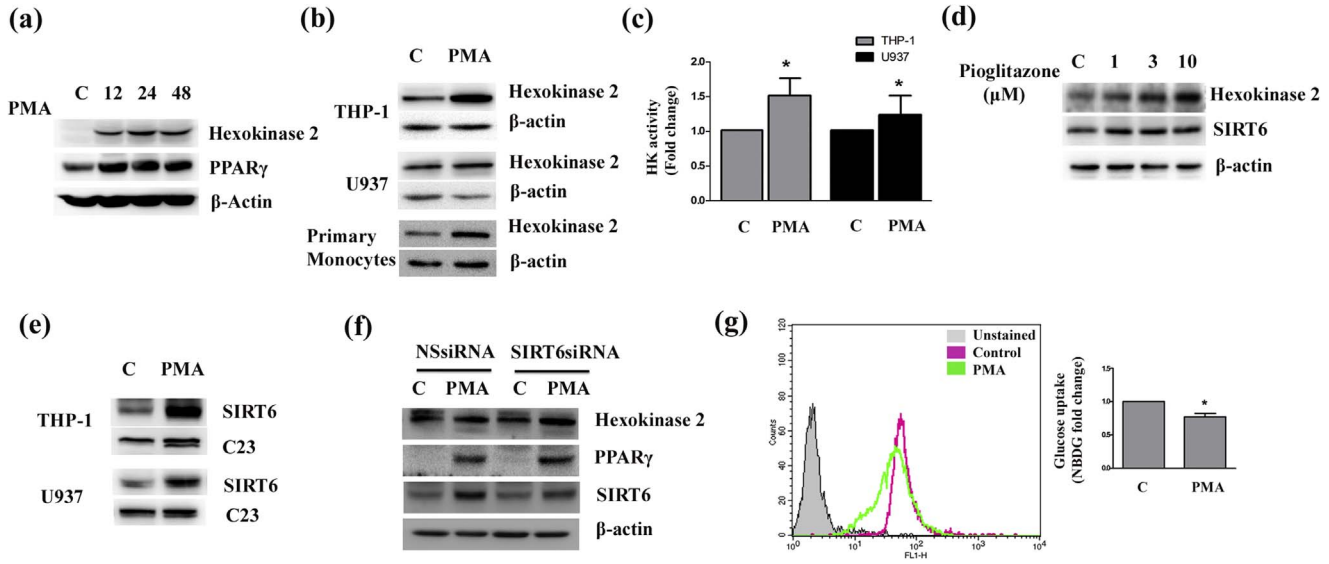

Fig. 1. PMA induced SIRT6 negatively regulates HK2 expression. (a) Time dependent increase in HK2 and PPARγ upon PMA treatment in THP-1 as shown by Western blot. (b) PMA induces HK2 expression in THP-1, U937 and primary human monocytes as depicted by Western blot. (c) Increased levels of HK activity in THP-1 and U937 cells treated with PMA as demonstrated by colorimetric HK activity assay. Graph represents HK activity, expressed as fold change over control. (d) Western blot depicts a dose dependent increase in HK2 and SIRT6 upon treatment of THP-1 cells with PPARγ agonist Pioglitazone. (e) Western blot indicating increased SIRT6 levels in monocytic cell lines treated with PMA for 48 h. (f) siRNA mediated SIRT6 knockdown increases HK2 and PPARγ expression upon PMA treatment. Knock-down efficiency of SIRT6 siRNA is shown. (g) Reduced glucose uptake in THP-1 cells upon PMA induced monocyte differentiation. Representative histogram shows NBDG fluorescence intensity in control and PMA treated THP-1 cells. Graph represents NBDG fluorescence intensity, expressed as fold change over control. Blots (a, b, d, e, f) are representative of three independent experiments. β-actin or c23 levels are shown to establish equivalent loading. Graphs represent data as means ± S.E.M. pooled from at least three experiments. *Significant change from control ($P < 0.05$).

2. Materials & methods

2.1. Cell culture and treatment

Human monocytic THP1 and U937 cells were cultured in RPMI 1640 medium supplemented with 100 μg/ml penicillin and streptomycin, 1% sodium pyruvate, 1% sodium glutamate, 50 μM 2-ME, 50 μM HEPES, and 10% heat-inactivated FBS (Life Technologies, Carlsbad, CA). After 4 h of serum starvation, cells were pre-treated with 100 μM HK2 inhibitor (Lonidamine, Tocris Bioscience, Bristol, UK), 20 nM autophagy inhibitor (Bafilomycin A1, Sigma Aldrich,St. Louis, MO, USA), 50 μM Arp2 inhibitor (CK666, Tocris Bioscience, Bristol, UK) and 50 μg/ml CXCR4 inhibitor (AMD 3100, Tocris Bioscience, Bristol, UK) for 2 h and then stimulated with 200 nM PMA for 48 h. Cells were then processed for subsequent studies. All reagents were purchased from Sigma-Aldrich,St. Louis, MO, USA unless otherwise stated.

2.2. Monocyte isolation and ethics statement

Peripheral blood mononuclear cells (PBMC) were isolated using Ficoll/Hypaque 1.077 g/ml as previously described by cell culture flask adherence or plastic adherence method [20]. For monocyte isolation by adherence, 10–15 × 10^6 PBMC per flask were seeded into T25 cell culture flasks, and allowed to adhere in a 5% CO_2 incubator at 37 °C for 2 h in 5 ml of serum-free culture media. Non-adherent cells were removed and the adherent cells were carefully washed twice with medium. Blood samples were collected in accordance with the guidelines of the institutional human ethics committee of National Brain Research Centre.

2.3. Western blot analysis

Protein isolated from untreated and treated cells was electrophoresed on 6–12% polyacrylamide gel and Western blot was performed as described previously [21], using antibodies against HK2, β-catenin, p-VASP, Arp2, Cofilin, p-JNK, JNK (Cell Signaling, Danvers, MA, USA); LC3-II, (Abcam, Cambridge, UK); Chibby, VDAC and Bax (Santa Cruz, CA, USA); TIGAR, SIRT6 (Novus Biological, Cambridge Science Park, Cambridge, UK) and CXCR4 (R & D Systems, Inc., Minneapolis, USA). Secondary antibodies were purchased from Vector Laboratories Inc. (Burlingame, CA, USA). After addition of enhanced chemiluminescent reagent (Millipore, Billerica, MA, USA), blots were exposed to Chemigenius Bioimaging System (Syngene, Frederick, MD, USA) for developing and images were captured using Genesnap software (Syngene, Frederick, MD, USA). The blots were stripped and reprobed with anti-β-actin (Sigma-Aldrich, St. Louis, MO, USA) or c23 (Santa Cruz, CA, USA) to determine equivalent loading as described previously [21].

2.4. Co-immunoprecipitation

Immunoprecipitation was performed with whole cell extracts (500 μg) obtained from THP1 cells treated with PMA. Protein extracts were incubated with 2 μg of anti-VDAC antibody (Santa Cruz, CA, USA)