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PARP1 facilitates EP300 recruitment to the promoters of the subset of RBL2-dependent genes

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ABSTRACT

Differentiation of human monocytes is associated with proliferation arrest resulting from activation of the inter alia retinoblastoma protein family of gene repressors, which target gene promoters in an E2F-dependent manner. To investigate RBL2 contribution to defining monocyte phenotype and function, we used primer libraries. We identified genes encoding two surface receptors (*CXCR1* and *IL17RE*) and two TLR signaling mediators (*CD86* and *NFKB2*) that are repressed by the RBL2-E2F4-HDAC1-BRM complex. Surprisingly, PARP1 co-regulated 24 out of the 28 identified genes controlled by RBL2. Upon RBL2 silencing, PARP1 was recruited to one subset of RBL2-dependent genes, represented by *MAP2K6* and *MAPK3*. RBL2 silencing also restored *PARP1* transcription. Gene promoters enriched in PARP1 were characterized by increased histone acetylation and the replacement of HDAC1 with EP300. While PARP1 was dispensable for HDAC1 dissociation, EP300 was found only at gene promoters enriched in PARP1. EP300 activated transcription of PARP1/RBL2 co-regulated genes, but not genes solely controlled by RBL2. DNA was a prerequisite to the formation of an immunoprecipitated PARP1-EP300 complex, suggesting that PARP1 enabled EP300 binding, which in turn activated gene transcription. Notably, PARP1 overexpression failed to overcome the inhibitory effect of RBL2 on *MAP2K6* and *MAPK3* transcription. The same interdependence was observed in proliferating cancer cells; the low abundance of RBL2 resulted in PARP1-mediated EP300 recruitment to promoters of the *MAP2K6* and *MAPK3* genes. We conclude that RBL2 may indirectly regulate transcription of some genes by controlling PARP1-mediated EP300 recruitment.

1. Introduction

The acquisition of cell identity during differentiation of most cell types is associated with cell cycle exit and induction of a novel gene expression program, which leads to the elaboration of a specialized phenotype and function [1]. Restriction of the cell cycle in response to internal and external stimuli is executed by hypophosphorylation of retinoblastoma transcriptional co-repressors. Retinoblastoma transcriptional co-repressors associate with E2F transcription factors and bind to the promoters of E2F-dependent genes encoding inter alia core players of cell cycle machinery [2]. The members of the retinoblastoma family (RB1/p105, RBL1/p107 and RBL2/p130) show some selectivity to particular E2Fs, as well as to the mode of growth inhibition (therefore also some redundancy). The recruitment of histone remodeling enzymes or complexes, such as HDAC1, SUV39H1, SWI/SNF, and PRC2, is governed by the inhibitory effects of retinoblastoma proteins on gene expression. The activity of these histone-remodeling enzymes results in chromatin compaction [3,4]. Since E2F-responsive promoters go beyond genes controlling cell cycle progression, the role of retinoblastoma proteins, particularly in differentiation-associated transcriptional reprogramming, has been expanding [5,6]. In hematopoietic lineage development, pRb promotes monocyte commitment to $CD34^+$ hematopoietic stem and progenitor cells in a dual way: by interacting directly with hematopoietic transcription factors, including CEBPB, SPI1, ELF1,

Abbreviations: iHDAC, Inhibitor of histone deacetylases; iEP300, Inhibitor of E1A-associated cellular p300 transcriptional co-activator protein; iPARP1, Inhibitor of poly(ADP-ribose) polymerase 1; CHX, cycloheximide; iDNMT, DNA methyltransferase; shCTRL, Cell line stably transfected with control vector, expressing basal level of PARP1; shPARP1, Cell line stably transfected with vector carrying shRNA for PARP1, expressing low level of PARP1; pCMV3-EMPTY, Human monocytes transfected with control vector, expressing basal level of PARP1; pCMV3-PARP1, Human monocytes transfected with vector carrying cDNA for PARP1, expressing higher level of PARP1

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and by repressing factors that drive neutrophil differentiation [7]. Although RBL2-E2F4/5 complexes accumulate in some terminally differentiated, post-mitotic cells, nothing is known about the contribution of RBL2 to defining the physiological function of human monocytes.

According to our recent findings in human monocytes, the RBL2-E2F4-HDAC1-BRM complex represses expression of poly(ADP-ribose) polymerase 1 (PARP1). PARP1 is involved in the regulation of a wide range of intracellular processes, and, under certain conditions, acts as a co-factor for gene transcription [4,8]. By directly associating with some histone writers and erasers and by ADP-ribosylating chromatin-interacting proteins (NFκB, NFATc1, YY1, KDM5B, Spt16, ISWI, nucleosomes), PARP1 determines, both positively and negatively, the ability of genetic regulatory elements to interact with the transcriptional machinery [9–13]. The downregulation of PARP1 transcription was also observed during the differentiation of other cell types, such as myoblasts into myotubes, suggesting that high expression of PARP1 and/or ADP-ribosylation interferes with differentiation into myotubes and monocytes and/or their proper functioning [4,14]. Bearing in mind that monocytes constitute an important component of the innate immune system, during their development the transcription program must provide monocytes with a specific panel of surface receptors for cytokines, chemokines, and pathogen-associated molecular patterns, as well as with components of intracellular signaling cascades (such as Toll-like receptor pathway). The monocyte transcription program must also simultaneously repress genes encoding proteins characteristic for other cell types and corresponding precursors [15,16]. Previous reports on PARP1 involvement in cell development indicate that this enzyme is capable of controlling expression of stemness factors (*SOX2*, *NANOG*, *ZFP42*), as well as up and down regulation of cell type specific genes (NFATc1, endoderm-specific genes) [17–20]. However, the complex nature of documented interactions makes PARP1 effects on a particular gene transcription dependent on the cell type and local chromatin conformation.

In this manuscript, we address two fundamental questions. First, do RBL2-based repressive complexes directly determine the pattern of monocyte receptors and TLR downstream signaling in human monocytes? Second, to what extent does RBL2 define monocyte phenotype by repressing *PARP1*? Using gene silencing and bioinformatic approaches, we identified genes independently controlled by RBL2 and by PARP1 in blood-derived monocytes. Surprisingly, we identified numerous groups of genes co-regulated by both RBL2 and PARP1. Importantly, we describe a new RBL2/PARP1/EP300 axis, which controls gene transcription regardless of the cell type. PARP1 was found to be critical for the activation of a subset of RBL2/PARP1-dependent genes when RBL2 is silenced. RBL2 silencing also restored *PARP1* transcription. PARP1 binding to the promoters of RBL2/PARP1-dependent genes enabled recruitment of EP300 in human monocytes. PARP1 binding to the promoters of RBL2/PARP1-dependent genes also maintained acetyltransferase associated with gene promoters in proliferating cancer cells, which actively transcribe *PARP1* as well as genes co-regulated by RBL2/PARP1. These results suggest a functional interplay between RBL2, PARP1, and EP300. Genome-wide analysis revealed a similar distribution of PARP1 and EP300 around transcription start sites and the co-occupancy of some gene promoters by PARP1 and EP300 in cancer cells.

2. Materials and methods

2.1. Materials

U937, HMC1, THP-1, A549 and MCF7 cell lines were from ATCC (USA). RosetteSep™ Monocytes Enrichment Cocktail was purchased from STEMCELL Technologies (Grenoble, France), and cell culture media were from Biowest (CytoGen, Zgierz, Poland). WB antibodies and other materials were purchased from the following manufacturers: anti-PARP1 (sc-8007), anti-α-tubulin (sc-5546), siPARP1 (sc-29437), PARP-1 shRNA plasmid (sc-29437-SH), control shRNA Plasmid-A (sc-108060), and puromycin were from Santa Cruz Biotechnology (AMX, Łódź, Poland); TRI Reagent, iHDAC (sodium butyrate), iDNMT (5-aza-cytidine), CHX (cycloheximide), anti-E2F4 antibody (05-312), anti-rabbit IgG (A0545), and anti-mouse IgG (A4416) (whole molecule)–-peroxidase antibody produced in goat were from Sigma Aldrich (Poznan, Poland); the ChIP grade antibodies, anti-histone H3 (#4620), anti-H3K4me3 (#9751), anti-PARP1 (#9532), anti-RBL2 (#13610), and normal rabbit IgG (#2729) were purchased from Cell Signaling Technology (LabJOT, Warsaw, Poland); anti-acetyl-histone H3 (Lys9 + Lys14) (PA5-16194), anti-HDAC1 (PA1-860), anti-EP300 (PA1848), siRBL2 (#AM16708), Lipofectamine RNAiMAX, Dynabeads™ Protein G, High-Capacity cDNA Reverse Transcription Kit, SuperSignal™ West Pico Chemiluminescent Substrate, OptiMem, DNAse I were from Thermofisher Scientific (Warsaw, Poland). iEP300 (C646) was purchased from Cayman Europe, while Advanced TC™ culture plates from Greiner Bio-One (Biokom, Janki/Warsaw, Poland). Kapa Sybr Fast qPCR Master Mix was purchased from Kapa Biosystems (Polgen, Łódź, Poland). ViaFect™ Transfection Reagent was purchased from Promega (Warsaw, Poland), Human Cytokine and Chemokine Receptor Primer Library (HCCR-I) and Human Toll-like Receptor Signaling Primer Library (HTLR-I) from RealTime Primers (Prospecta, Warsaw, Poland).

2.2. Monocyte isolation and cell culture

Human monocytes were isolated using RosetteSep™ Monocytes Enrichment Cocktail from buffy coats derived from healthy donors in a Blood Bank in Lodz. The study, as well as the processing of buffy coat and human-derived monocytes, was approved by the Bioethical Committee at the University of Lodz (no 19/KBBN-UŁ/I/2016), and all methods were performed in accordance with the relevant guidelines and regulations. Freshly isolated cells were allowed to attach (Advanced TC™ culture plates, Geiner Bio-One) for 3 h prior to further processing. Monocytes, THP1, and U937 cells were cultured in RPMI, A549 and MCF7 cells in DMEM, and HMC1 in IMDM. All media was supplemented with 10% FBS and penicillin/streptomycin solution (50 U/ml and 50 μg/ml, respectively).

2.3. Gene silencing

For permanent PARP1 silencing, A549 and MCF7 cell lines were transfected with lentiviral vector plasmids (control: shCTRL and PARP1 targeting: shPARP1). In brief, vectors (0.1 μg) were mixed with ViaFect™ transfection reagent (0.6 μl) in OptimMEM. After incubation at room temperature for 20 min, DNA-lipid complexes were transferred to 100,000 cells cultured in DMEM full medium. Selection with puromycin (1 μg/ml) was started 48 h after transfection.

For transient gene silencing, cells were transfected with siRNA. Briefly, siRNA:Lipofectamine RNAiMAX complexes (0.6 nmol siRNA per 1 μl of transfection reagent) were prepared in serum free OptiMEM medium and added to cells (500,000) cultured in full RPMI growth medium.

2.4. Transient PARP1 overexpression

Isolated monocytes were transfected with pCMV3-EMPTY and pCMV3-PARP1 expression vectors using ViaFect™ Transfection Reagent. In brief, 0.1 μg DNA was mixed with 0.8 μl ViaFect™ Transfection Reagent in OptiMem, incubated for 15 min at room temperature, and added to 200,000 freshly isolated monocytes. After 24 h, cells were treated with iEP300 for another 48 h. RNA was extracted with TRI Reagent and treated with DNA-free™ DNA Removal Kit prior to reverse transcription.