Molecular Immunology 67 (2015) 482–491

Contents lists available at ScienceDirect

Molecular Immunology

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

Polycomb recruitment at the Class II transactivator gene

Nathaniel H. Boyd[a,1], Julie E. Morgan[a,1], Susanna F. Greer[b,*]

[a] Division of Cellular Biology and Immunology, Department of Biology, Georgia State University, Atlanta, GA 30302, United States
[b] Department of Biology, Georgia State University, Petit Science Center, 100 Piedmont Avenue, Suite 632, Atlanta, GA 30302-4010, United States

ARTICLE INFO

Article history:
Received 2 July 2015
Accepted 4 August 2015
Available online 15 August 2015

Keywords:
EZH2
JARID2
YY1
CIITA
Polycomb recruitment

ABSTRACT

The Class II Transactivator (CIITA) is the master regulator of Major Histocompatibility Class II (MHC II) genes. Transcription of CIITA through the IFN-γ inducible CIITA promoter IV (CIITA pIV) during activation is characterized by a decrease in trimethylation of histone H3 lysine 27 (H3K27me3), catalyzed by the histone methyltransferase Enhancer of Zeste Homolog 2 (EZH2). While EZH2 is the known catalytic subunit of the Polycomb Repressive Complex 2 (PRC2) and is present at the inactive CIITA pIV, the mechanism of PRC2 recruitment to mammalian promoters remains unknown. Here we identify two DNA-binding proteins, which interact with and regulate PRC2 recruitment to CIITA pIV. We demonstrate Yin Yang 1 (YY1) and Jumonji domain containing protein 2 (JARID2) are binding partners along with EZH2 in mammalian cells. Upon IFN-γ stimulation, YY1 dissociates from CIITA pIV while JARID2 binding to CIITA pIV increases, suggesting novel roles for these proteins in regulating expression of CIITA pIV. Knockdown of YY1 and JARID2 yields decreased binding of EZH2 and H3K27me3 at CIITA pIV, suggesting important roles for YY1 and JARID2 at CIITA pIV. JARID2 knockdown also results in significantly elevated levels of CIITA mRNA upon IFN-γ stimulation. This study is the first to identify novel roles of YY1 and JARID2 in the epigenetic regulation of the CIITA pIV by recruitment of PRC2. Our observations indicate the importance of JARID2 in CIITA pIV silencing, and also provide a novel YY1-JARID2-PRC2 regulatory complex as a possible explanation of differential PRC2 recruitment at inducible versus permanently silenced genes.

1. Introduction

A chromatin modifying complex currently under extensive study is the Polycomb Repressive Complex 2 (PRC2). PRC2 is a four-protein complex consisting of core members Embryonic Endoderm Development protein (EED), Suppressor of Zeste 12 (SUZ12), Enhancer of Zeste Homolog 2 (EZH2), and Retinoblastoma Associated Protein (RbAp46/48). EZH2 is the mammalian homolog of *Drosophila* Enhancer of Zeste [E(z)] [1,2] and is responsible for catalyzing an addition of trimethyl group on lysine 27 of histone H3 (H3K27me3) in human cells [3–5]. SUZ12 binds to EZH2, stabilizes its methyltransferase activity, and is required for the PRC2 complex to function [6]. EED stabilizes the complex by binding to the product of PRC2 catalysis, H3K27me3, and creating a positive feedback loop by further stimulating the complex [7]. RbAp46/48 is a histone chaperone known to bind histones H3 and H4, which further stabilize the complex [8]. In addition to the four core subunits, more controversial alternate subunits have been described to play roles in PRC2 regulation named Polycomb-like proteins 1, 2 and 3 (PCL1, PCL2, PCL3) and AEBP2 [6,9]. These proteins have been described to effect PRC2 function in a tissue-specific manner.

DNA binding domains among the PRC1 and PRC2 proteins have yet to be identified in any species [7,10]. In *Drosophila*, both PRC complexes are recruited to chromatin targets by DNA elements termed Polycomb Response Elements (PREs), but these PREs are not commonly found on mammalian DNA, with only two recent reports describing mammalian PREs [11,12]. Importantly, these mammalian PREs contain Yin-Yang 1 (YY1)-binding motifs; a zinc-finger protein which has been shown to both activate and repress gene transcription [13,14] and is related to the PRE-binding protein in *Drosophila,* Pleiohomeotic (PHO) [15–17]. In

Abbreviations: PRC2, polycomb repressive complex 2; EZH2, enhancer of zeste homolog 2; JARID2, jumonji, AT rich interactive domain 2; YY1, Yin-Yang 1; CIITA pIV, class II transactivator promoter IV; MHC, major histocompatibility complex; lncRNA, long non-coding RNA; HOTAIR, HOX antisense intergenic RNA; IFN-γ, interferon gamma; HOX, homeobox gene; Pho, pleiohomeotic; H3K27me3, histone H3 lysine 27 trimethylation; H3K4me3, histone H3 lysine 4 trimethylation; EED, embryonic ectoderm development; SUZ12, suppressor of zeste 12; RbAp46/48, retinoblastoma associated protein 46/48; E(z), enhancer of zeste; PRE, polycomb response element; HLA, human leukocyte antigen; APC, antigen presenting cell.

* Corresponding author at: Fax: 404 413 5301.
E-mail addresses: nboyd3@gsu.edu (N.H. Boyd), jmorgan30@gsu.edu (J.E. Morgan), sgreer@gsu.edu (S.F. Greer).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.molimm.2015.08.003
0161-5890/

addition, Jumonji/ARID domain-containing protein 2 (JARID2) has recently been shown to be a member of the PRC2 complex and regulates PRC2 binding to target genes in human embryonic stem cells [18–22]. By studying the roles of YY1 and JARID2 in PRC2 recruitment, we hope to elucidate novel mechanisms by which PRC2 is recruited to target promoters.

In the current study we focus on MHC Class II (MHC II), which are molecules responsible for presentation of peptides from extracellular origin and inducibly expressed on all nucleated cells in the presence of inflammatory cytokines, particularly interferon-γ (IFN-γ) [23–25]. MHC II molecules are important drivers of adaptive immune responses as they bind to $CD4^+$T cells, and initiate inflammatory responses to extracellular antigens. MHC II expression is tightly regulated at the level of transcription by a master regulator, the Class II Transactivator (CIITA) [26]. In the absence of CIITA, MHC II fails to transcribe, resulting in a loss of MHC II expression [27]. Because CIITA controls the expression of a universally required immune molecule, it has four distinct promoters that are active in different cell types. Here we focus on promoter IV, which is responsible for inducible CIITA expressed through IFN-γ induction in all nucleated cells [28,29].

Understanding PRC2 and EZH2 involvement in transcriptional control of CIITA promoter IV (CIITA pIV) is of significance to human health. Decreased expression of MHC Class II expression on tumor cells has long been a proposed mechanism of tumor immune evasion. MHC Class II expression has been shown to be decreased in breast cancer, B-cell lymphoma, and Hodgkin lymphoma, which may correlate with increased EZH2 binding [30–33]. Artificially inducing MHC II expression in tumor cells results in high levels of endogenous antigen presentation and elevated immunogenicity, thus suggesting therapeutic avenues [34,35]. Here we detail the binding patterns of two putative PRC2 recruitment proteins, YY1 and JARID2, and their effects on the expression of the inducible gene CIITA pIV. Our findings demonstrate YY1 and JARID2 as novel binding partners to recruit and regulate PRC2 binding and the activity involved in regulating CIITA expression.

2. Materials and methods

2.1. Cells

HeLa (human epithelial) cells from ATCC (Manassas, VA) were cultured and passaged in Dulbecco Modified Eagle Medium (DMEM) (Mediatech, Inc., Herndon, VA) containing 10% Fetal Bovine Serum (FBS), 5 mM L-glutamine, and 5 mM penicillin-streptomycin at 37 °C and 5% carbon dioxide.

2.2. Antibodies

Antibodies recognizing MYC-tag, FLAG-tag, and JARID2 were purchased from Abcam (Cambridge, MA). Antibodies recognizing YY1, HA-tag, and HRP conjugated rabbit antibody were purchased from Santa Cruz (Santa Cruz, CA). Antibody recognizing H3K27me3 was purchased from Active Motif (Carlsbad, CA). Antibody recognizing EZH2 was purchased from Cell Signaling Technology (Danvers, MA) and HRP conjugated mouse antibody was purchased from Promega (Madison, WI).

2.3. Plasmids

The Myc-EZH2-pCDNA3 plasmid construct was described previously and was kindly provided by Jer-Tsong Hsieh [36]. The pCMV-HA-JARID2 construct was described previously [21]. Flag-YY1 was described previously and was kindly provided by Myra Hurt [37]. The CIITA pIV luciferase construct was described previously and was kindly provided by Bernard Mach [38]. The pcDNA control plasmid was described previously [39].

2.4. Primers

Primers and probes for CIITA pIV and for the GAPDH promoter used in ChIP assays are as previously described [40–42] as well as primers and probes for CIITA pIV mRNA, HLA-DRA mRNA and GAPDH mRNA [41,42]. Primers and the probe for JARID2 mRNA expression are as follows: JARID2 sense CACCGTCCTCCCCAATAACA, JARID2 antisense CTCGAGACCAGCATGAGGTAGA, and JARID2 probe 6 FAM-AGGGTCCATCCTGCGTCACCTCG-TAMRA.

2.5. siRNA Constructs and transient transfections

Short interfering RNA duplexes (siRNA) pre-designed for YY1, JARID2 or scrambled All Star Control (Qiagen) were used to knock down expression of YY1 or JARID2. HeLa cells were transfected with 10 nM of YY1 or JARID2 specific siRNA or All Star scrambled control sequence according to the HiPerfect Transfection Reagent protocol (Qiagen). Cells were lysed in NP40 cell lysis buffer with EDTA free complete protease inhibitor (Roche) and were analyzed by western blot or RNA expression levels for efficiency and specificity of knock down.

2.6. Dual luciferase assay and transient transfections

HeLa cells were plated at 1×10^5 cells/well in 6-well tissue culture plates. Twenty-four hours after plating, cells were transfected with 150 ng of plasmid (CIITA-Luc, Flag-YY1, HA-JARID2, Myc-EZH2, or pcDNA control) using Genejuice transfection regent (Novagen) according to manufacturer's protocol. Each sample was also transfected with renilla luciferase as a control for expression levels. 24 h after transfection, cells were treated with 500 U/mL IFN-γ and harvested 6 h after stimulation as indicated. Cells were lysed using 1× lysis reagent (Promega), supplemented with protease inhibitor, scraped from plates, and a dual luciferase was run per manufacturer's protocol.

2.7. Co-immunoprecipitation assay and transient transfections

HeLa cells were plated at a cell density of 8×10^5 in 10 cm tissue culture plates. Cells were allowed to adhere to plate overnight. Cells were then transfected with 5 μg of plasmid (Flag-YY1, HA-JARID2, Myc-EZH2, or pcDNA control) using GeneJuice (Novagen) per manufacturer's protocol. Cells were harvested 18 h later in NP40 lysis buffer with EDTA free complete protease inhibitor (Roche). Lysates were centrifuged, normalized for protein concentration, and precleared with protein G beads (Pierce). After preclear, lysates were immunoprecipitated overnight with anti-Myc, anti-HA, or anti-Flag agarose beads (Sigma) or protein G mock bead control. For endogenous Co-IP, lysates were incubated with anti-JARID2 antibody (Abcam) overnight, and the following morning incubated with Protein G beads for 1 h. Bead-immune complexes were washed with dilute NP40 to remove background and subsequently eluted from beads and denatured by boiling for 5 min in Laemmli gel loading buffer with beta-mercaptoethanol (Bio-Rad). The collected supernatant was analyzed by western blot.

2.8. Chromatin Immunoprecipitation (ChIP)

HeLa cells were plated at a density of 2×10^6 in 10 cm tissue culture plates. Cells were treated with IFN-γ (500 U/mL) as indicated. After IFN-γ stimulation, cells were cross-linked with 37% formaldehyde. Crosslinking was stopped with 0.125 M glycine and cells were harvested and lysed in cell lysis buffer (5 mM PIPES, 85 mM KCl)