Transcriptional and Epigenetic Regulation of the Type III Interferon Response

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ABSTRACT

The type III interferons (IFN-λ1, 2, 3; alternatively known as IL-29, 28A, and 28B respectively) play a critical role in controlling virus replication in specific tissues, including the lung and gut. These cytokines activate an antiviral response though a distinct receptor (IFN-λR) that consists of two differentially regulated subunits: IFN-λRα and IL-10Rβ. The IFN-λRα subunit is unique to IFN-λR and is expressed in a cell-type specific manner. Tissue-specific IFN-λRα mRNA expression is important for the biological activity of the cytokine, which is thought to primarily protect epithelial cells from specific viral pathogens. Furthermore, IFN-λ1 has shown promise in clinical trials as immunotherapy for chronic HCV infection because it reduces virus replication in the liver without causing hematological side effects that are associated with IFN-α regimen. This characteristic is believed to be partially due to the restricted expression of IFN-λ receptor. Despite the importance of IFN-λR expression both in the natural host antiviral response and for the potential clinical use of this cytokine family, almost nothing is known about how the receptor expression is regulated. We hypothesize that tissue-specific transcription factors and epigenetic modifications contribute to the differential expression of IFN-λ receptor in various cell types. Our current data supports our hypothesis in that we identified a region within IL28RA (the gene that encodes IFN-λRα) promoter that potentially contains negative transcription regulators. In addition, we also demonstrated that DNA methylation and histone acetylation are involved in the regulation of IFN-λ receptor expression. Finally, inhibition of DNA methyltransferase (DNMT) and histone deacetylase (HDAC) activities led to increase in the level of IFN-λRα expression in low expressing cells, further linking chromatin structure with receptor expression. These studies lay the groundwork for understanding how the IFN-λ response is influenced by other host- or pathogen-encoded regulatory factors, and may also identify new ways to harness IFN-λ activity for therapeutic purposes.

BACKGROUND

Interferons (IFNs) are a group of indispensable cytokines that play extremely important roles in the immune system. IFN-λ1, IFN-λ2, IFN-λ3, collectively known as the type III interferons, are newly identified interferons that demonstrated antiviral activity against a variety of viral pathogens. Different from the type I IFNs including IFN-α and IFN-β, IFN-λ binds to its unique receptor, IFN-λ receptor, which is a heterodimer composed of IFN-λRα (encoded by *IL28RA*) and IL-10Rβ (encoded by *IL10RB*). Previous studies have shown that the single nucleotide polymorphisms (SNPs) in *IL28B*, the gene that encodes IFN-λ3, strongly correlate with the clinical outcome of chronic Hepatitis C virus (HCV) infection. Currently, recombinant IFN-λ1 is also in Phase 2 clinical trial for chronic HCV patients, further stressing its crucial role in the innate immunity. However, how IFN-λ and its receptor is regulated remains elusive. In contrast to the ubiquitous expression of IL-10Rβ, IFN-λRα is differential expressed among various cell types (**Figure 1**). Compared to neurons and astrocytes which have relatively low level of IFN-λ receptor expression, cells of epithelial origin, such as A549, Huh7, HepG2 and primary hepatocytes have high expression level. The underlying mechanism that results in this differential expression is still unclear.

Gene expression is a multi-step process that can be regulated by many factors. In the context of chromatin, the binding affinity of RNA Polymerase II is determined by the combination of transcription factors, DNA methylation status, histone modifications, et cetera (**Figure 2**). We are currently studying the contribution of transcription factors and epigenetic modifications in regulating IFN-λ receptor expression in different cell types.

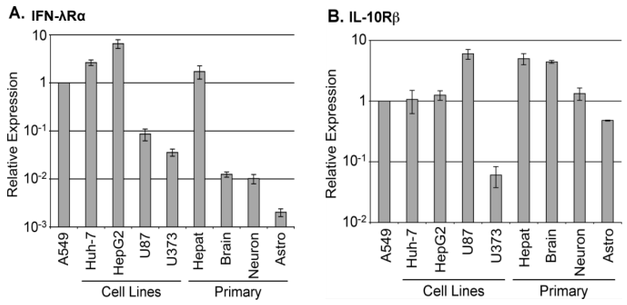

Figure 1. Relative mRNA expression level of IFN-λRα and IL-10Rβ in different cell types. (A) The expression level of IFN-λRα was measured by RT-qPCR using total RNA extracted from A549 (lung epithelial carcinoma cell line), Huh7 (hepatoma cell line), HepG2 (hepatocellular carcinoma cell line), U87 (astrocytoma cell line), U373 (astrocytoma cell line), primary hepatocytes, brain cells, neurons and astrocytes. **(B)** The expression level of IL-10Rβ was determined by RT-qPCR using total RNA extracted from the same cell lines and primary cells.

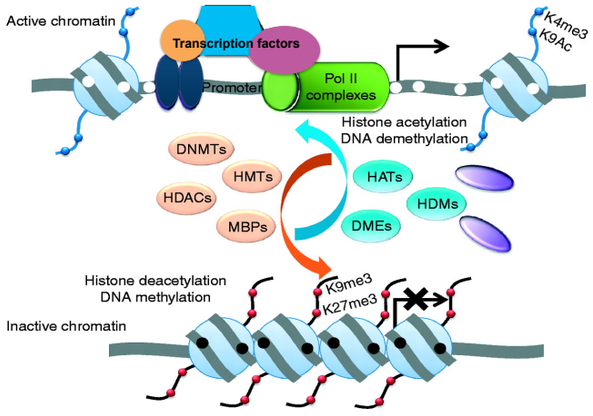

Figure 2. Transcription factors and epigenetic modifications are two major mechanisms that regulate gene expression. The histone tails are sensitive to posttranslational modifications, which contribute to chromatin status. Moreover, hypermethylation of promoter is associated with transcriptional silencing due, in part, to the loss transcriptional factors and accessibility by the transcriptional machinery as represented by RNA Pol II complexes. The inactive chromatin has increased affinity for methylated DNA-binding proteins (MBPs), which further recruit histone deacetylases (HDACs), DNA methyltransferases (DNMTs), and histone methyltransferases (HMTs). Methylated promoters are associated with unique repressive histone markers, which include trimethylation of histone 3 (H3) lysine (K) 9, and H3K27. In contrast, unmethylated promoters have more affinity for histone acetylases (HATs), histone demethylases (HDMs), DNA demethylases (DMEs), and histone marks associated with active chromatin, including acetylated H3-K9 and trimethylated H3-K4. Me, histone methylation; Ac, histone acetylation; black filled circle, methylated CpG dinucleotides; white filled circle, unmethylated CpG dinucleotides. (Adapted from Zhang X, Ho S, *J Mol Endocrinol* 2011;46:R11-R32)

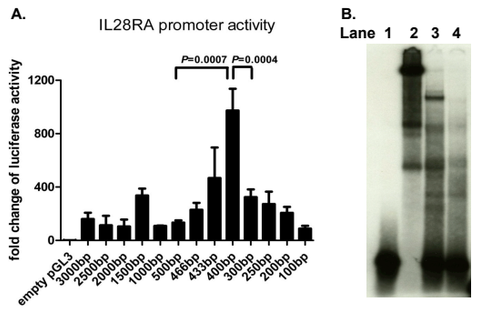

Figure 3. IL28RA promoter contains a segment that is potentially bound by negative transcription factors. (A) A series of fragments containing different length of IL28RA promoter were inserted into basic pLG3 vector. U373 were co-transfected with each of these plasmids plus pRL-TK vector which encodes renilla luciferase to serve as internal control. The cells were harvested 48 hours post transfection and the lysates were subject to dual-glo luciferase assay. Firefly luciferase activity was normalized using renilla luciferase readout. **(B)** EMSA probe containing -434~-401 region of IL28RA promoter was end-labeled with ^{32}P and incubated with nuclear extracts isolated from U87 cells. Lane 1 (probe alone); lane 2 (probe + nuclear extracts); lane 3 (probe + nuclear extracts + poly (dA:dT)); lane 4 (probe + nuclear extracts + poly (dA:dT) + cold competitor). A gel-shift was observed in lane 3 and disappeared in lane 4 in the presence of excessive unradiolabeled probe, suggesting that the band is due to specific interaction.

RESULTS

In order to identify the transcription factors that regulate IFN-λRα expression, the promoter region of *IL28RA*, the gene that encodes IFN-λRα, was PCR amplified and different length was inserted into basic pGL3 vector upstream of firefly luciferase open reading frame. Due to the lack of eukaryotic promoter sequence in basic pGL3 vector, the promoter activity of different fragments can be measured by luciferase activity. As is shown in **Figure 3A**, there is a dramatic increase in luciferase activity when -500~-400 region was removed, indicating the potential binding of transcription repressors. Similarly, there is a significant decrease in luciferase activity when -400~-300 was deleted, implying the presence of transcription activators. A series of ^{32}P-labeled probes containing different regions of -500~-400 of IL28RA promoter were used in electrophoretic mobility shift assay (EMSA) to further narrow down the binding of negative regulators. A specific interaction was observed between the -434~-401 probe and nuclear extracts from U87 cells with low receptor expression level (**Figure 3B**).

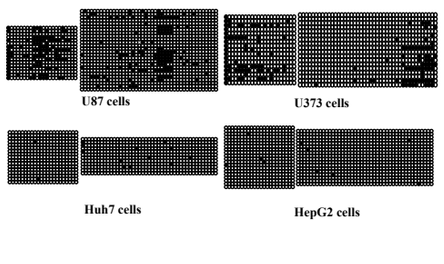

Figure 4. DNA methylation study of CpG islands within IL28RA promoter using bisulfite conversion sequencing. Unmethylated cytokines are converted to uracils under bisulfite treatment while methylated cytokines are protected from the conversion. Each dot represents one CpG dinucleotide; filled dots for methylated ones and open for unmethylated ones. Each row represents an individual clone of the population. For each cell type, square on the left stands for CpG island I; square on the right stands for CpG island II.

Bioinformatic prediction reveals two putative CpG islands within IL28RA promoter, which may be subject to DNA methylation. Using bisulfite conversion sequencing, we showed that the CpG islands in IL28RA promoter were hypermethylated in U87 and U373 cells, both of which are low expressing cells whereas they were hypomethylated in Huh7 and HepG2 cells, both of which are high expressing cells (**Figure 4**). In addition, by using chromatin immunoprecipitation (ChIP), we demonstrated that acetylated histone 3 and RNA polymerase II, both of which are markers for active transcription, were enriched in IL28RA promoter in Huh7 cells while H3K27me3, the hallmark of repressed gene expression, was enriched in IL28RA promoter in U87 cells (**Figure 5**). This indicates that IL28RA is in an open chromatin structure in the high expressing cells and in a repressed chromatin structure in the low expressing cells. Furthermore, a significant increase in IFN-λRα expression was observed in U87 cells in the presence of DNMT and HDAC inhibitors (**Figure 6A**). Importantly, the combination of inhibitor treatment renders U87 cells much more sensitive to IFN-λ stimulation, converting low expressing cells to high expressing cells (**Figure 6B**).

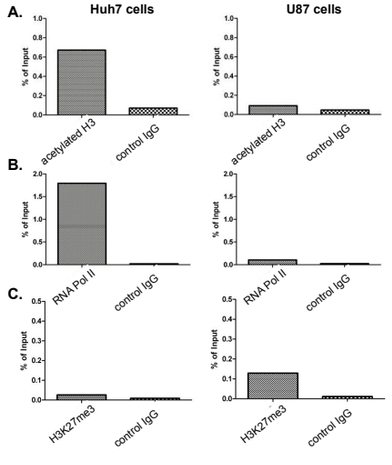

Figure 5. Chromatin immunoprecipitation (ChIP) performed on IL28RA promoter in Huh7 and U87 cells. (A) Chromatin was obtained from Huh7 and U87 cells by crosslinking and sonication. Sonicated chromatin was incubated with magnetic beads in presence of either antibody against pan-acetylated histone 3 or control rabbit IgG. DNA was extracted after extensive wash and reversal of crosslinking and subject to quantitative PCR analysis using specific primers for IL28RA promoter. **(B)** ChIP was performed using antibody against RNA polymerase II. **(C)** ChIP was performed using antibody against H3K27me3.

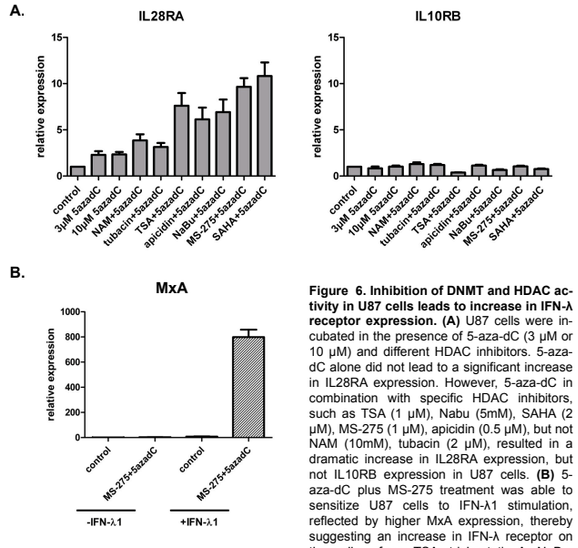

Figure 6. Inhibition of DNMT and HDAC activity in U87 cells leads to increase in IFN-λ receptor expression. (A) U87 cells were incubated in the presence of 5-aza-dC (3 μM or 10 μM) and different HDAC inhibitors. 5-aza-dC alone did not lead to a significant increase in IL28RA expression. However, 5-aza-dC in combination with specific HDAC inhibitors, such as TSA (1 μM), Nabu (5mM), SAHA (2 μM), MS-275 (1 μM), apicidin (0.5 μM), but not NAM (10mM), tubacin (2 μM), resulted in a dramatic increase in IL28RA expression, but not IL10RB expression in U87 cells. **(B)** 5-aza-dC plus MS-275 treatment was able to sensitize U87 cells to IFN-λ1 stimulation, reflected by higher MxA expression, thereby suggesting an increase in IFN-λ receptor on the cell surface. TSA, trichostatin A; NaBu, sodium buty-rate; NAM, nicotinamide; SAHA, suberoylanilide hydroxamic acid.

SUMMARY

- -500~-400 region of IL28RA promoter is potentially bound by transcription repressors while -400~-300 region is regulated by transcription activators.
- EMSA reveals an interaction between -434~-401 region and nuclear extracts from low expressing cells.
- CpG island in IL28RA promoter is hypermethylated in low expressing cells while hypomethylated in high expressing cells.
- An “open” or relaxed chromatin state is observed for IL28RA promoter in Huh7 cells in contrast to a “closed” or condensed chromatin state in that of U87 cells.
- IFN-λ receptor expression increases in U87 cells in the presence of DNMT and HDAC inhibitors.
- U87 cells become more sensitive to IFN-λ stimulation under 5-aza-dC plus MS-275 treatment.

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