Integrative analysis of -omics data reveals estrogen-responsive regions in Th17 and Treg genomes

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Motivation
Each cell has a specific genomic regulatory landscape that control gene expression and define cell identity. The genomic regulatory landscape is composed of regulatory elements like enhancers and promoters. Specific epigenetic modifications contribute to gene expression in different ways, thus the existence of a combinatorial histone code define the function of each genomic region. Super Enhancers (SE) are clusters of enhancers collectively bound by transcription factors (TF) that drive transcription of genes involved in cell identity. CD4+ T cells have the capability to respond to changing environmental stimuli repolarizing towards alternative cell fates. Epigenetic reprogramming is the series of events underpinning this phenotypic plasticity, and this process can make a difference between a pro-inflammatory and an anti-inflammatory environment. Therefore CD4+ T cell plasticity is critical for proper immune cell homeostasis and host defense, but can become deleterious, driving autoimmunity such as Multiple Sclerosis (MS). T helper 17 (Th17) cells are pro-inflammatory and can induce autoimmune disease, on the contrary regulatory T (Treg) cells have a regulatory phenotype and maintain immune homeostasis. Hence, Th17/Treg balance is a mirror of inflammation in MS and is strictly connected with disease outcome. A reduction of relapse rates in pregnant MS patients supports the hypothesis of estrogens as immune response regulators. Estrogens promote the activation of Estrogen Receptor alpha (ER) and its transcriptional activity through interactions with specific genomic elements called Estrogen Response Element (ERE). These elements are the downstream target of cytokine pathways and this mechanism is pursued through TF binding. CD4+ T cells genomic regulatory regions may be considered as control platform for immune response and their identification and characterization is a strong challenge.
Methods
Here, we exploited public epigenomic data from Roadmap Epigenome Project and 25 public RNA-Seq datasets performed on Th17, Treg cells, a pool of T helper cells and their precursors Naïve T cells. In order to perform an integrative analysis of CD4+ T cells epigenome and transcriptome we developed a computational strategy summarized in Figure 1. Specifically, epigenomics data were used to predict Super Enhancer (SE) by Rank of Super Enhancers (ROSE) algorithm using as input

ChIP-Seq data of the epigenetic mark of active enhancers, acetylated lysine 27 of histone 3. Then, active epigenetic domains were selected using a 200 bp human genome segmentation with the corresponding predicted functional annotation by ChromHMM, a tool based on imputed data for 12 epigenetic marks and Hidden Markov Model. The overlap of these domains with SEs identified the main set of Cell type Specific regulatory Regions (CSR) that define cell identity. Transcriptomic data were used to identify differentially expressed lineage-specific transcription factors (TFs) by DE-Seq R package. Finally, an individual motif-based sequence analysis was performed by FIMO package of MEME-suite within CSRs in order to reconstruct regulatory networks.

Results

Collectively, histone modification enrichment within CSRs showed cell type specificity among CD4+ T cell subtypes. Network reconstruction resulted in four and ten CSR-associated TFs that compose the human regulatory networks for the specification of Th17 and Treg cells, respectively. The occurrence of Estrogen Response Elements (ERE) in CSRs suggested Estrogen Receptor alpha (ERα) binding at selected regions, revealing putative ERα-responders in these cells. Experimental data confirmed that CSRs have susceptibility in terms of epigenetic variations and ERα binding in human PBMCs from pregnant and post-partum MS patients and also in 17b-estradiol (E2) treated in-vitro polarized Th17 cells. Altogether these data suggest that estrogens orchestrate pregnancy immunotolerance acting on CD4+ T cells epigenome.