Chapter: 2

The epigenetic regulation of IL-10 production in tumor-induced Treg cells and other T cell subsets.
Introduction

The ability of transcription factors to bind to DNA at regulatory regions on any gene is affected by their concentration, post-translational modifications and subcellular localization, as well as by the state of the chromatin and underlying DNA. The epigenetic context in which transcription factors function is provided by the position and compaction of nucleosomes, the interactions of nucleosomes with the DNA, post-translational histone modifications and the methylation status of the DNA (Lee et al, 2006; Berger, 2007; Kouzarides, 2007). Therefore, unlike genetic information, epigenetic information is not encoded by changes in the sequence of the DNA, but by differential methylation of the DNA and modifications of chromatin, which affect whether, when and to what level specific genes are expressed in a given cell. Because the DNA sequence remains unchanged, epigenetic modifications and the information that they encode can be heritable but plastic — the potential to erase modifications and inscribe new ones is retained. The fact that distinct signals are capable of driving naïve Th cell differentiation in-vitro into well-defined subsets makes CD4+T cell activation a useful model for understanding how epigenetic regulation can influence cellular differentiation and fate determination. The differentiation of T helper cells is intricately coupled to epigenetic changes in chromatin structure, histone and DNA modifications, and the expression of small noncoding RNAs (Ansel et al, 2003; Merkenschlager and Wilson, 2008; Wilson et al, 2009). Earlier analysis of epigenetic changes during T helper cell differentiation has largely focused on the gene loci encoding cytokines that mark Th1 or Th2 cell lineages, i.e., IFNγ and IL-4, IL-5 and IL-13, respectively. Recent technological advances, e.g., Chromatin immunoprecipitation (ChIP) followed by genomic tiling arrays (ChIP-chip) or by high-throughput sequencing (ChIP-Seq), have allowed genome-wide examination of histone modifications and DNA methylation that accompany changes in gene expression in CD4+ T cell subsets. Such analysis has provided insights into the mechanisms underlying T helper cell plasticity (Wei et al, 2009).

Tri-methylation of histone H3 lysine 4 (H3K4me3) is a permissive mark found in the promoters and the enhancers of active genes, whereas tri-methylation of histone H3 lysine 27 (H3K27me3) is present in broad domains that encompass inactive genes. H3K4me3 and H3K27me3 modifications are both present in some genomic regions, and these bivalent modifications have been suggested to poise genes ready for either activation or repression during differentiation. The epigenetic marks found at the IFNγ, IL-4, and IL-17 gene loci correlated precisely with Th1 and Th2 cell lineages, with H3K4me3 at the IFNγ locus and H3K27me3 at the IL-4 and IL-17 loci in Th1 cells and H3K4me3 at the IL-4 locus and H3K27me3 at the IFNγ and IL-17 loci in Th2 cells. Intriguingly, the Tbx21 (encoding T-bet) and Gata3 genes displayed a bivalent status in Th2 and Th1 cells, respectively, consistent with a report suggesting that interconversion can occur between Th1 and Th2 cells under appropriate inflammatory conditions.
(Krawczyk et al, 2007). Consistent with the plasticity evident for Treg cells, Tbet, Gata3, and RORγt were all bivalently modified in Treg cells. Such bivalent modifications may allow specific lineage regulator gene loci to be activated under different polarizing conditions, thus reprogramming Treg cells into other lineages. Similarly, in vitro differentiated Th17 cells displayed bivalent epigenetic status at the Tbet and GATA3 loci, and such a result suggests that these cells are pre-conditioned to be redirected toward Th1 or Th2 cell fates. Such reprogramming appears to be unidirectional, given that repressive epigenetic configuration was observed at both the IL-17a and RORγt loci in Th1 and Th2 cells (Wei et al, 2009). Thymus-derived nTreg cells and peripherally derived iTreg cells appear to differ in their propensity for reprogramming. This is reflected in the DNA methylation status of the Foxp3 locus and in histone marks at the RORγt locus. H3K27me3 was found at the IL-17a locus, whereas H3K4me3 was found at the RORγt locus in iTreg cells, consistent with the notion that RORγt expression was induced by TGF-β, but the RORγt-directed IL-17 expression was inhibited by FOXP3 (Wei et al, 2009; Zhou et al, 2008a). In contrast, in nTreg cells, bivalent H3K4me3 and H3K27me3 were present at the RORγt locus, allowing for potential coexpression of FOXP3 and RORγt. All together, these data on epigenetic modification of histones and DNA highlight the plasticity of CD4+ T cell differentiation programs, suggesting that the “lineage-specific” transcription factors may not necessarily have mutually exclusive expression patterns. Lineage specification is therefore not only determined by the epigenetic status of signature cytokine loci but also relies on the epigenetic states of key transcription factors. Like lineage specific transcription factor epigenetic instabilities at regulatory region also control the expression of cytokine genes. With current technological advances, it is now possible to unravel epigenetic profile of T helper cell lineages, including the analyses of further histone modifications and of multiple target gene loci associated with the individual lineages. Unlike other IFNγ, IL-4 and IL-17 expression of IL-10 not restricted to the FOXP3+ Treg cells only. Th1, Th2, Th17 and Tr1 cells may also produce variable amounts of IL-10 but the epigenetic regulation of this cytokine gene in different T cell subsets is still not fully understood. Therefore, understanding the epigenetic regulation of IL-10 transcription in FOXP3+ Treg cells and other FOXP3+ T cell subsets will open new therapeutic intervention strategies to perturb their immunosuppressive activities. Expression of cytokine genes solely depends on activation and interaction of master transcription factor with other associated partners as well as epigenetic factors on gene regulatory regions (promoter, enhancer etc.). Genome-wide analysis of FOXP3-binding sites coupled to gene-expression profiling suggests that FOXP3 can act as both a transcriptional activator and a repressor (Marson et al, 2007). For example, binding of FOXP3 to the 5’ regulatory regions of CTLA4 and IL2Ra (CD25) results in their activation, whereas interacting of FOXP3 to the IL-2 and IL7RA promoters facilitates their repression (Zheng et al, 2007; Gavin et al, 2007). Like transcription factors FOXP3 may associate with several chromatin modifying
partners such as TIP-60, HDAC1, 2, 4, 7 that transactivates or represses target genes epigenetically. Similar to lineage specific transcription factor, distinct STAT proteins are also responsible for maintaining the global epigenetic landscape of different T cell subsets. As STAT family transcription factors are critical for sensing the cytokine milieu and promoting helper T cell differentiation, it seemed logical to consider that they might contribute to the distinctive modifications seen in helper cell subsets. However, the extent to which STAT proteins are drivers or followers of epigenetic modifications in T cells was by no means clear, and in fact, initial studies analyzing IFNγ-signaling and STAT1 implied that epigenetic changes largely preceded STAT1 binding (Robertson et al, 2008). The first issue to tackle was to define STAT target genes in helper cell subsets. Though it has widely been accepted that STATs are critical mediators of gene transcription, transcriptional profiling did not distinguish between directly and indirectly regulated genes (Levy and Darnell, 2002). There appear to be subsets of genes that are highly dependent on STATs for promoting both gene expression and the local epigenetic environment. One of the particular interest is a subset of genes that are bound by STAT4 in Th1 cells but by STAT6 in Th2 cells, for which the two STATs have opposing effects on local epigenetic patterns. All these observations indicate that both transcription factor and STAT partners are essential to drive transcription of cytokine genes by recruiting chromatin modifiers in regulatory region as well as maintain global epigenetic signature to sustain heritability of CD4+ T cell subsets.

**Aims and Objectives**

I. To identify any chromatin-modifying factor that may associate with IL-10 transcription and maintain epigenetic signature in tumor-associated Treg cells.

II. To check whether FOXP3 or STAT3 may recruit any epigenetic factor at IL-10 promoter region.

III. To unravel the association of other transcription factor that may responsible to drive IL-10 transcription in FOXP3+ T cell subsets.

IV. To determine the role of other STAT proteins in FOXP3+ T cell subsets.

V. To unravel the alteration of epigenetic status at IL-10 promoter in FOXP3+ Treg cells and other FOXP3- T cell subsets.
Results

2.1. Localized chromatin modification by FOXP3-associated HAT1 induces STAT3-mediated IL-10 transcription in Treg cells.

In search of chromatin modifiers that associated with FOXP3-STAT3 complex and may recruit at IL-10 promoter to relax it epigenetically we re-identify other spots of 2-D gel electrophoresis. Our data distinctively indicated that apart from STAT3 (spot#1), FOXP3 was also associated with HAT1 or histone acetyl transferase-1 (spot#3). Next, we attempted to unveil the mechanism supporting the cotranscriptional role of FOXP3 in regulating the transcriptional activity of STAT3. Our co-immunoprecipitation studies further confirmed the direct association of HAT1 with FOXP3 in tumor-associated Treg cells. This experiment validates that apart from STAT3 as a transcription activator of IL-10, HAT1 may be a crucial factor for the IL-10 induction in tumor-associated Treg cells (Fig.-2.1).

Figure-2.1. FOXP3 also interacted with epigenetic modifiers HAT1 in Treg cells. (a) MALDI-TOF MS analysis of spot#3 obtained after 2D gel electrophoresis (left) for FOXP3-associated proteins are represented in tabular form after the processed peaks. (b) FOXP3 and HAT1-associated proteins were immunopurified from the nuclear extract of FOXP3- and STAT3-silenced Treg cells and then immunoblotted with HAT1 and pY-STAT3 antibodies. Histone H1 was used as internal control (c) Ribbon diagram of FOXP3 dimer and HAT1 complex.
2.2. **FOXP3-recruited HAT1 induces histone acetylation and enhances STAT3 transcription activity.**

HAT1 is type-B histone acetyltransferase which has long been presumed to participate in the synthesis of newly synthesized histone during the process of chromatin assembly and localized only in the cytoplasm. Several studies later confirmed that HAT1-containing complexes are clearly found in both the cytoplasm and the nucleus, and, in fact, appear to be predominantly nuclear. In our study, we also found that in tumor Treg cells HAT1 also translocate into the nucleus along with FOXP3. HAT1 acetylates DNA-bound histone to relax the chromatin structure as required for accessibility of transcription factors to their respective DNA-binding sites, we next checked the histone acetylation status of the *IL-10* promoter region in these cells. Results showed that in FOXP3- or HAT1-silenced cells, the histone acetylation was decreased significantly in the *IL-10* promoter region, whereas STAT3 silencing partially reduced the histone acetylation. FOXP3 was found to be bound to HAT1 and helped it to translocate from the cytosol into the nucleus where a transient ternary complex was formed with STAT3 that bound the *IL-10* promoter, resulting in epigenetic modification (Fig.-2.2).

![Figure-2.2. FOXP3 recruited HAT1 in nucleus to relax IL-10 promoter epigenetically. A) In STAT3, FOXP3, HAT1, or p300 siRNA-transfected conditions, tumor-associated Treg cells were analyzed for histone acetylation pattern of IL-10 promoter. B) Confocal images of normal T cells and tumor-associated Treg cells immunostained with specific FOXP3 and HAT1 antibodies.](image-url)
2.3. HAT1 recruits to the STAT3 binding site and acetylates specific histone lysine residue to relax the IL-10 promoter epigenetically

In comparison to normal T cell intense level of HAT1 was found to bind in the IL-10 promoter region of Treg cells. HAT1 acetylated histone H4 at K5 and K12 residues predominantly and histone H2A at K5 residue moderately (Fig. 2.3). These modifications led to the opening of the STAT-responsive element of the IL-10 promoter, thereby facilitating the docking of STAT3-FOXP3 complex with consequent transcription of IL-10. The IL-10 promoter binding activity of HAT1 in tumor-associated Treg cells was determined by ChIP assay. Specific histone acetylation pattern of STAT-binding sites of the IL-10 promoter were determined by ChIP assay with acetylated histone H2AK5, H4K5, H4K8, and H4K12 antibodies.

![Figure 2.3](image)

**Figure-2.3.** HAT1 acetylates specific histone residues and initiate IL-10 transcription. (a) Specific histone acetylation pattern of STAT-binding sites of IL-10 promoter was determined by ChIP assay with acetylated histone H2AK5, H4K5, H4K8, and H4K12 antibodies. (b) IL-10 promoter binding activity of HAT1 in tumor-associated Treg cells was determined by ChIP assay.

2.4. FOXP3 acts as a co-transcription of STAT3 in tumor-induced T-regulatory cells

HAT1 acetylates DNA-bound histone to relax the chromatin structure as required for accessibility of transcription factors to their respective DNA-binding sites, we next checked the histone acetylation status of the IL-10 promoter region in these cells. FOXP3 was found to be bound to HAT1 and helped it to translocate from the cytosol into the nucleus where a transient ternary complex was formed with STAT3 that bound the IL-10 promoter, resulting in epigenetic modification. HAT1 acetylated histone H4 at K5 and K12 residues predominantly and histone H2A at K5 residue moderately. These modifications led to the opening of the STAT-responsive element of the IL-10 promoter, thereby facilitating the docking of STAT3-FOXP3 complex with consequent transcription of IL-10. A similar kind of predictive docking module with target-receptor specificity study showed the probable association between dimeric-FOXP3 and HAT1. As soon as HAT1 modifies IL-10 promoter, a FOXP3-
dimer forms a complex with dimeric-STAT3. The probable competitive phenomenon happens because the FOXP3-STAT3 complex is formed with an increase of surface area for a more favorable interaction, as compared to FOXP3-HAT1. These findings indicate that FOXP3, in association with HAT1, modifies the IL-10 promoter epigenetically, providing a conformational space to the STAT3-FOXP3 complex on its putative binding site (Fig.-2.4). These results identify FOXP3 as a major transcription cofactor of STAT3 in mediating the IL-10 transcription in Treg cells in tumor milieu.

Figure-2.4. FOXP3 acts as a cotranscription factor in Treg cells. A predictive docking module with target-receptor specificity study showed that FOXP3 dimer binds HAT1 and brings it into the nucleus to form a transient ternary complex with the STAT3 dimer. This complex epigenetically modifies the IL-10 promoter, thereby making space for the docking of the STAT3-FOXP3 complex.

2.5. Generation of different T cell subsets by in-vitro T cell polarization.

Naïve CD4+CD45RA+ T cells interacted with antigen presented by definite antigen presenting cells (dendritic cells, macrophages) and in the presence of specific cytokine milieu can differentiate into various T-helper cell subsets. We have isolated naïve CD4+CD45RA+ T cells by negative magnetic selection from the cord blood of female healthy volunteers with prior informed consent. The naïve T
cells then cultured in serum free RPMI medium supplemented with lineage specific cytokine factors and/or neutralizing antibodies. After 4 days mRNAs were isolated and the transcript levels were checked by Real-Time PCR.

Figure-2.5. In-vitro T cell Polarization with lineage specific cytokines. Naïve CD4+CD45RA+ T cells were cultured under Th0, Th1, Th2, Th-17, or iTreg conditions for 4 days. Transcript levels of (a) lineage specific transcription factors and (b) cytokines were analyzed by qPCR.

2.6. FOXP3+ T cells also produce IL-10 in different circumstances.

IL-10 is a pleiotropic cytokine initially thought to secrete only by Th2 cells. But, later it was found that other T cell subsets like Th1, Treg, Th17 and Tr1 cells also produces IL-10 at different circumstances. Naïve T cells (CD4+CD45RA+) were isolated from umbilical cord blood by negative magnetic bead isolation system and then incubated with distinct cytokine condition to polarize them into different T cell subsets. All T cell subsets (Th1, Th2, Treg, and Th17) generated by in-vitro produced variable
amounts of IL-10 confirm that its expression not restricted to FOXP3+ Treg cell only. Tr1 cells, which are FOXP3+, but expresses intense levels of IL-10 isolated from Hepatitis-B patients.

![Flow cytometry plots showing IL-10 production by different T cell subsets](image)

**Figure 2.6. IL-10 production by different T cell subsets.** CD4+CD45RA+ T cells were polarized into different T cell subsets after incubating with lineage specific cytokine cocktails and neutralized antibodies. Flowcytometric density plot depicted that all T cell subsets produced diverse levels of IL-10.

### 2.7. STAT and c-MAF proteins are inevitable for IL-10 production in FOXP3+ T cell subsets.

Our results showed that like tumor Treg cells, other T cell subsets such as Th1, Th2, Th17 and Tr1, can also secrete IL-10 via STAT-mediated signaling. Although all T cell subsets secrete IL-10, its transcription depends on respective lineage specific STAT proteins: STAT4 for Th1, STAT6 for Th2 and STAT3 for Treg, Th17 and Tr1 (Fig. 2.7a). Silencing of STAT protein diminished the level of IL-10 transcription prove that they are essential for IL-10 synthesis. However, it is interesting to note that Th1, Th2, Th17 and Tr1 cells drive IL-10 transcription without the requirement of FOXP3. Therefore, in these T cell subsets IL-10 was produced in the FOXP3 independent manner. Similar to STAT proteins IL-10 transcription in these cells depends on cMAF protein, which is present in relatively low levels in Treg cells. Like STAT proteins silencing of cMAF perturbs IL-10 production in FOXP3+ T cell subsets whereas cMAF is not responsible for IL-10 transcription in FOXP3+ Treg cells. Therefore, both STAT and cMAF proteins are essential for IL-10 expression in FOXP3+ T cell subsets, but the silencing of STAT proteins inhibits cMAF production indicates that cMAF transcription depends on levels of STAT proteins (Fig. 2.7b). These results prove that in FOXP3+ T cell subsets IL-10 transcription initiated through STAT mediated cMAF induction.
Both STAT and cMAF are essential for IL-10 transcription in FOXP3+ T cell subsets. In Th1, Th2, Th17 and Tr1 cells knockdown of either STAT (4, 6 & 3) or cMAF reduced IL-10 expression; however, in tumor induced Treg cells only STAT3 knockdown, but not cMAF, reduced IL-10 expression (Fig. 2.7a). In these cells, STAT knockdown completely abrogates cMAF expression (Fig. 2.7b).

2.8. STAT3 in FOXP3+ Treg cells and cMAF in FOXP3- T cell subsets activate IL-10 transcription by binding at promoter region

The IL-10 transcription in T cell subsets is regulated by cMAF and STAT proteins. In tumor associated Treg cells STAT3 along with FOXP3 occupy at the distinct STAT binding site at IL-10 promoter to accelerate the transcription process, whereas in other T cell subsets cMAF binds to cMAF binding site to continue IL-10 transcription. Chromatin-Immunoprecipitation (ChIP) coupled with Real-Time PCR at both STAT binding site and cMAF binding site confirmed that STAT3 and cMAF are evitable for IL-10 transcription in FOXP3+ Treg cells and other T-cell subsets respectively. The responsiveness of two distinct regions in promoter region clearly indicate that there might be some epigenetic factors associated with these regions that switching the entire IL-10 transcription process.

STAT3 in Treg cells and cMAF in other T cell subsets recruited to the IL-10 promoter and initiates transcription process. ChIP assay showed that in tumor Treg cells STAT3 binds to STAT-responsive element, whereas in Th1, Th2, Th17 and Tr1 cells cMAF binds to its cognate binding site of the IL-10 promoter. There is no cMAF binding in tumor Treg cells or STAT binding in other T cell subtypes and no cross-binding.
2.9. STAT binding region of IL-10 promoter region hyper-acetylated and de-methylated in FOXP3+ Treg cells.

In tumor associated Treg cells, FOXP3 associated with HAT1 hyper-acetylates histone H2A and H4 of STAT-responsive element in de-methylated IL-10 promoter to make a pocket for STAT3-FOXP3 binding to its cognate site to induce IL-10 expression. The H2AK5 residue acetylated moderately whereas H4K5 and H4K8 residue acetylated predominately in Treg cells confirms that HAT1 is essential to relax IL-10 promoter epigenetically. In Th1, Th2, Th17 and Tr1 cells H2AK5, H4K5 and H4K8 residue remain inactive clearly confirmed that HAT1 not involve in other T cell subsets to acetylates and relax IL-10 promoter epigenetically. The cytoplasmic HAT1 translocate into nucleus and induces acetylation at STAT3 promoter binding site to activate IL-10 transcription. The positive ChIP signals at HAT1 specific histone residues in STAT-binding site further confirmed that this site remain epigenetically active and accessible to bind FOXP3-STAT3 complex to resume IL-10 transcription. To determine whether STAT-binding region of the IL-10 promoter, become the de-methylated where HAT1 get access to prompt the transcription process we performed methyl-DNA immunoprecipitation. Our result confirms that in Treg cells STAT3-binding region IL-10 promoter is hyper-acetylated by HAT1 as well as de-methylated, necessary to continue the IL-10 transcription (Fig.-2.9). On the other hand, in other T cell subsets STAT-binding promoter region becomes methylated and condensed where no HAT1 activities were found.

![Figure 2.9. Epigenetic alteration of STAT-binding site in IL-10 promoter.](image)

(a) Amongst all T cell subtypes only in tumor induced Treg cells histone H2A (at K5 site) and H4 (at K5 & K12 sites) are acetylated at STAT-binding site of IL-10-promoter. (b) On the contrary, in all other T cell subtypes in the STAT-binding site of IL-10-promoter is DNA-methylated.

2.10. Alteration of epigenetic signature in IL-10 promoter region in FOXP3+ Treg cells and FOXP3- T cell subsets

It is now evident that either STAT3 along with FOXP3 or STAT mediated induction of cMAF bind to a distinct promoter binding region to activate the IL-10 transcription process. Our results stress the fact that STAT-binding region apart from FOXP3+ Treg cells becomes condensed and de-methylated. As
transcription of \( IL-10 \) depends on two distinct promoter regions we further checked permissive and repressive histone modification as well as DNA methylation status in both STAT-binding region and cMAF binding region of all T cell subsets. It is interesting that H3K4Me3 permissive mark was found both STAT- and cMAF-binding region and remains unchanged. The repressive H3K27Me3 mark was found in cMAF-binding site and absent in STAT-binding site in Treg cells, whereas it is present in STAT-binding site and absent in cMAF-binding site in other T cell subsets. The presence of a permissive histone methylation mark (H3K4Me3) and shifting of repressive histone methylation mark (H3K27Me3) of STAT- and cMAF-binding site indicates that both regions are bivalent and poised. In addition, we also found permissive histone acetylation mark H3K27Ac of both STAT- and cMAF-binding site that shows an inverse relationship between H3K27Me3. Presence of H3K27Ac and absence of H3K27me3 at particular promoter-binding site and vice versa strengthen our prediction that the IL-10 promoter is bivalent and poised in both regions. Association of DNA methylation binding protein MeCP2 in promoter region further confirms that in Treg cell and other T cell subsets alternatively used STAT3-binding site and cMAF-binding site respectively.

**Figure-2.10. Histone modification and DNA methylation in STAT and cMAF promoter-binding region controls IL-10 transcription.** Permissive histone methylation (H3K4Me3), Repressive histone methylation, permissive histone acetylation (H3K27Ac) and DNA methylation (MeCP2) pattern were checked by ChIP assay in STAT and cMAF responsive elements in Treg cells and other T cell subsets (Th1,Th2,Th17 and Tr1).
Discussion

It is now acknowledged that in addition to the transcription factor binding, a crucial part of gene regulation is the epigenetic regulation which includes DNA methylation, ATP-dependent nucleosome remodeling and histone modifications (Jaenisch and Bird, 2003). Acting together with transcription factors, these chromatin modifications have major effects on gene expression. The fact that distinct signals are capable of driving naïve Th cell differentiation in-vitro into well-defined subsets makes CD4+ T cell activation a useful model for understanding how epigenetic regulation can influence cellular differentiation and fate determination (Russ et al, 2013). Comparison of the epigenetic profiles of signature effector gene loci within Th1 and Th2 cells has been particularly informative. In response to Th1 differentiation signals, the IFNγ locus of naïve T cells is remodeled to a permissive epigenetic signature that reinforces and heritably maintains IFNγ gene expression in the long-term. At the same time, the IL-4 locus is remodeled to have a repressive epigenetic signature resulting in the shutdown of IL-4 gene expression (Russ et al, 2013). Recent work using ChIP-Seq has been instrumental in providing genome level insights into how epigenetic processes might regulate helper T cell fate selection. For instance, genome-wide comparison of H3K4me3 and H3K27me3 distribution in naive, Th1, Th2, and Th17 cells, combined with global transcriptional profiling demonstrated that the distribution of just two histone modification (H3K27me3 and H3K4me3) could provide a simple explanation for the differences in phenotypes observed amongst these different T cell subsets. For example, upon differentiation from a naïve T cell state into the various effector helper T cell subsets, H3K4me3 deposition was observed at signature effector gene loci within distinct helper T cell subsets (e.g., IFNγ in Th1, IL-4 in Th2, and IL-17 in Th17) (Lee et al, 2009). Moreover, H3K27me3 deposition was correlated with transcriptional shutdown of effector gene loci that are characteristic of other helper T cell subsets. The epigenetic bivalency in CD4+ helper T cell subsets is considered a mechanism for poising gene loci for rapid activation or repression, can maintain some level of functional plasticity despite lineage commitment. It is tempting to speculate that this provides the immune system with inherent flexibility, allowing the redirection of pathogen-specific Th-responses. Unlike IFNγ, IL-4 and IL-17, IL-10 is produced at variable amounts in all T cell subsets like Th1, Th2, Treg, Th17 and Tr1. Although the biological function of IL-10 in the immune system has extensively been studied for decades, little information is available on the molecular mechanism of its transcriptional regulation, especially at the chromatin level. As our studies confirmed that FOXP3 and STAT3 are crucial for IL-10 transcription in tumor induced Treg cells and STAT induced cMAF is essential to drive IL-10 induction in other T cell subsets. Previous reports have revealed that the transcription factor FOXP3 can dramatically change histone modifications at its binding loci and regulate expression of the adjacent genes (Chen et al, 2006).
In that case, FOXP3, having direct promoter occupancy, functions as a scaffolding molecule which helps in recruiting the different components of the transcriptional machinery (Chen et al, 2006). Several studies confirmed that in addition to the interaction with a number of transcriptional factors, FOXP3 also interacts with enzymes such as HAT and HDAC that regulate the activity of FOXP3 at the post-translational level. Earlier studies confirmed that histone acetyltransferase (HAT) TIP60 and histone deacetylase HDAC7 interact with the N-terminal of FOXP3 and increase its suppressive transcriptional activity (Lozano et al, 2013). TIP-60 promotes the acetylation of FOXP3, which enhances the suppressive transcriptional activity of FOXP3, but TIP-60 may also play a role in regulating FOXP3 activity independent of its HAT activity. The N-terminal region of FOXP3 is required for TIP60-FOXP3, HDAC7-FOXP3 association, as well as for the transcriptional repression of FOXP3 via its FKH domain (Xiao et al, 2010, Lozano et al, 2013). Binding of TIP60 with FOXP3 may regulate the oligomerization status of FOXP3 and therefore its DNA binding. Moreover, TIP60 may also recruit additional factors required for transcriptional repression/activation. Our attempt to explore the role of FOXP3 in aiding to STAT3-binding to IL-10 promoter revealed that besides STAT3, FOXP3 was also associated with HAT1 with these Treg cells. On binding to HAT1, FOXP3 brought the latter into the nucleus to form a transient ternary complex with STAT3. Since the molecular selectivity of FOXP3 was more biased towards STAT3 dimer, FOXP3-STAT3 binding freed HAT1 which then modified the IL-10 promoter epigenetically to provide a conformational space to STAT3-FOXP3 complex at its putative binding site. Interesting to note that like tumor-associated Treg cells, other T cell subsets such as Th1, Th2, Th17 and Tr1 can also secret IL-10 via STAT-mediated signaling. But the STAT-responsive element of the IL-10 promoter in these cells remained epigenetically silenced. So, failing to direct promoter occupancy, STATs (STAT4 in Th1 cells; STAT6 in Th2 cell, STAT3 in Th17 and Tr1 cells) induced cMAF, which docked the cMAF-responsive element in the promoter region to induce IL-10 expression. In tumor-associated Treg cells, however, FOXP3-associated HAT1 hyper-acetylated histone H2A and H4 of STAT-responsive element and the IL-10 promoter remained de-methylated to make a pocket for STAT3-FOXP3 binding to its cognate site. H3K4Me3 and H3K27Me3 profile at STAT-binding site and cMAF interacting domain in the IL-10 promoter region unravels the epigenetic regulation of pleiotropic cytokine IL-10 in different T cell subsets. The bivalent epigenetic modification and methylation studies at the promoter region indicate IL-10 gene regulates differently in FOXP3+ Treg cells and other T cell subsets. The transcriptional and epigenetic program in regulating IL-10 expression elucidates the functional plasticity of T cell subsets in different disease condition including cancer. Therefore, a greater understanding of the molecular mechanisms, and specific epigenetic mechanisms, that shape acquisition and maintenance of lineage-specific T cell function, will be key if we are to make advances in novel therapeutic strategies for a variety of diseases.