

Marking the 50th Anniversary of *Immunology*

Special regulatory T-cell review: FOXP3 biochemistry in regulatory T cells – how diverse signals regulate suppression

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Summary

FOXP3 is an acetylated and phosphorylated protein active in human regulatory T cells and forms oligomers which then associate with an even larger molecular complex. FOXP3 actively regulates transcription by recruiting enzymatic co-repressors and/or co-activators. FOXP3 complex ensembles are dynamically regulated by physiological stimuli such as T-cell receptor, IL-2 and proinflammation cytokine signals. Understanding the post-translational modifications of FOXP3 regulated by diverse signals and the biochemistry and structural chemistry of enzymatic proteins in the FOXP3 complex is critical for therapeutically modulating regulatory T cell function.

Keywords: acetylation; FOXP3; oligomerization; regulatory T cell; transcriptional repression

Two critical mechanisms need to be better understood in regulatory T-cell biology: (1) how FOXP3 is induced, and (2) how FOXP3 functions *in vivo*. While the level and duration of Foxp3 expression is essential and sufficient for regulatory T (Treg) cell function in mice,^{1,2} induced FOXP3 expression alone is not sufficient in humans.^{3–6}

FOXP3 represses various downstream effectors of the T-cell activation signal pathways, and may also activate certain genes in Treg cells.^{7,8} Although the mechanisms by which FOXP3 produces Treg-cell-mediated suppression of various immune cells are largely unknown, it is clear that the function of FOXP3 is dependent on its binding partners, including several key transcription factors and various corepressors and/or coactivators in regulatory T cells.⁹

We have recently identified that FOXP3 is an acetylated protein¹⁰ and an oligomeric component of a large molecular complex.^{11,12} FOXP3 actively represses transcription by recruiting enzymatic corepressors, including the histone acetyltransferase TIP60 and the class II histone acetyltransferases HDAC7 and HDAC9.¹⁰ Moreover, transcriptional factors including NF-ATc2 and FOXP1, and corepressors including TIP60, HDAC7 and HDAC9, cofractionate with nuclear FOXP3 in a low molecular

weight complex.¹¹ Other FOXP3-associated chromatin remodelling factors, including BRG1, Ku70/Ku80 and MBD3, cofractionate with nuclear FOXP3 in larger molecular weight complexes.¹¹ These data suggest that FOXP3-associated chromatin remodelling factors and enzymatic subunits may be dynamically affected by diverse signals. Moreover, these cofactors may exert non-overlapping functions in Treg-cell function.

The physiological function of FOXP3 *in vivo* also appears to extend beyond T cells.^{13,14} Hinz and colleagues¹³ found that transforming growth factor- β_2 -induced FOXP3 expression in pancreatic ductal adenocarcinoma cells could completely suppress naïve T-cell proliferation *in vitro*, suggesting a mechanism of immune evasion in pancreatic cancer. Zuo and colleagues¹⁴ found that FOXP3 deficits could increase *Her2/neu* expression in murine and human breast cancers.

These findings were of interest to us because our laboratory first described the *neu* gene.^{15,16} The observation by Zuo *et al.* extended from recognition that female scurfy mice that were heterozygous for *Foxp3* had increased numbers of breast tumours. Studies in human cells also identified a role for loss of FOXP3 in female breast cancers that overexpressed *Her2/neu*.¹⁴ How wild-type FOXP3 represses *Her2/neu* expression is currently

unclear. It will be of interest to determine if FOXP3 represses *Her2/neu* transcription in tumour cells by the same mechanism used in Treg cells. While in T cells FOXP3 gains activity by recruiting the TIP60/HDAC7 corepressor complex and by interacting with other transcriptional factors such as NF-ATc2,^{17,18} AML1/RUNX1,¹⁹ FOXP1^{12,20} or ROR α ,²¹ it is entirely possible that a different ensemble of cofactors is operative in breast cancer cells.

We have recently found that ectopically expressed FOXP3 down-regulates the activation of mitogen-activated protein kinase (MAPK) activity via direct molecular interaction in human T cells (Li and Greene, unpublished data). FOXP3 may suppress the downstream signalling network shared by *p185Her2/neu* and the T-cell receptor, such as the MAPK signal pathway and the phosphoinositide 3-kinase-v-akt murine thymoma viral oncogene homolog 1 (PI3K-AKT) signal pathway.

FOXP3 expression as a complex leads to Treg-cell-mediated suppression of various immune cells in a cell-cell contact-dependent or -independent manner. Previous data on suppression indicated a role for elaborated or released molecules^{22–24} and recent findings of a role for the heterodimeric haematopoietin formed by Epstein-Barr virus-induced gene 3 (*EBI3*) and the p35 subunit of interleukin-12,²⁵ with the new name IL-35,^{26,27} as a mediator of suppression support this notion. Moreover, we previously identified a dominant role of ultraviolet-radiation-modified antigen-presenting cells as potent inducers of T cells mediating suppression.²⁸ Antigen-presenting cells may gain the ability to induce Treg cells by changes in enzymes operative in amino acid metabolism. Other enzymes that are operative in antigen-presenting cell subsets that alter tryptophan²⁹ or arginine³⁰ may lead to the induction of Treg cells through undefined pathways.

Whether FOXP3 suppresses T-cell proliferation by modulating the T-cell metabolism³¹ is not known. Pericellular adenosine, generated by two FOXP3⁺ Treg-cell-expressed ectoenzymes, including adenosine triphosphate/adenosine diphosphate hydrolysing enzyme CD39 and ecto-5'-nucleotidase CD73 from extracellular nucleotides, has recently being found as a critical mediator of the immunosuppressional function of FOXP3⁺ Treg cells on the activated effector T (Teff) cells.³² Another recent study by Bopp and colleagues³³ showed that cyclic adenosine monophosphate represents an immunosuppressive mediator that may be transferred from FOXP3⁺ Treg cells directly to effector T cells via a junction between Treg cells and effector T cells that is yet to be visualized.

Our work examines the biochemistry of posttranslational modification and the ordered complex ensemble of FOXP3 on local chromatin. This chromatin-bound ensemble is dynamically regulated by physiological stimuli such as T-cell receptor and proinflammation cytokine signals. Pharmaceutical agents and natural products that alter the

function of the histone acetyltransferases, deacetylases and methyltransferases may have benefit in human patients suffering from autoimmune diseases and cancer. We have recently found that histone deacetylase inhibitors affect the posttranslational modification of Foxp3 proteins and can ameliorate certain autoimmune diseases such as experimental autoimmune encephalomyelitis and collagen-induced arthritis in small animal models. Understanding the basic chemistry of FOXP3-mediated transcriptional repression may yield therapeutics for humans.

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