REVIEW ARTICLE

Regulation of immune responses, apoptosis, and tumorigenesis by separate FOXP-3-dependent genes: Connection with clinical manifestations

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Recently, forkhead/winged-helix family box protein P3 (FOXP-3) was described as the main regulator of regulatory T cells' activity. This transcription factor has the ability to control the immunosuppressive response of regulatory T cells. FOXP-3 has binding sites for different genes specific for proteins with various important functions. In this article, selected FOXP-3-dependent genes with known functions were divided into two groups. The first group of genes has main immunoregulatory functions, and the second group has the ability to regulate apoptosis and tumorigenesis. Investigation of the functions of all FOXP-3-dependent genes opens perspectives for applications in different fields of basic and clinical research.
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Introduction

Regulatory T cells (Tregs) are a subpopulation of CD4$^+$ cells and primarily maintain the immune system homeostasis of the organism. These cells have a high immunosuppressive potential for both innate and adaptive immunities. Disturbance of the cellular homeostasis results in either self-reactive autoimmune aggression or immune deficiency. The investigation of T cells with suppressive activity began in 1970, when new subpopulation of T-lymphocytes with immunosuppressive properties was first described.[1] Since this initial observation, numerous articles characterizing T cells with immune regulatory properties have been published; however, the mechanisms inherent to the immune response in these cells have not been fully elucidated.

Forkhead/winged-helix family box protein P3 and immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome

The immune response that characterizes Tregs is realized through the action of the forkhead/winged-helix family box protein P3 (FOXP-3) (Scurfin). The functional activity of

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CD4$^+$CD25$^+$ Tregs depends on the high expression of FOXP-3 gene and protein. FOXP-3 is a homodimer consisting of a proline-rich domain at the N-terminus in addition to a C2H2 zinc finger and a leucine zipper located in the center of the amino acid sequence. This central domain participates primarily in protein—protein interactions, whereas the C-terminal end of FOXP-3 is a forkhead DNA-binding domain. FOXP-3 has regions that allow it to bind to approximately 700 genes and intergenically encoded microRNAs. In addition, FOXP-3 may have opposing effects on different genes, facilitating the transcription of some genes while repressing the transcription of others. Changes or defects in the coding sequence of the FOXP3 gene result in the development of different pathological conditions. Complete inactivation of FOXP3 gene because of a condition known as immuno-dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome. IPEX syndrome is very rare in humans and has only been observed in male patients. This syndrome is characterized by a number of complications, including diarrhea, a failure to thrive, eczema, atopic psoriasiform dermatitis, alopecia, hemolytic anemia, thrombocytopenia, neutropenia, hypo- or hyperthyroidism, lymphoadenopathy, Type 1 diabetes mellitus, and increased susceptibility to infections. Other clinical complications may include respiratory distress, bruising, hypocalcemia, hepatic parenchymal disease, cholestasis, hypertension, cardiomegaly, glomerulopathy, encephalopathy, and testicular atrophy. IPEX animal models show a phenotype characteristic of the scurfy mouse.[2] These animals have scaly skin, progressive anemia, thrombocytopenia, leucocytosis, lymphoadenopathy, various infections, diarrhea, gastrointestinal injury, cachexia, and hypogonadism in males, and they typically die within the first few weeks after birth. FOXP-3-related activation of Tregs has been demonstrated by the hypersensitivity of scurfy mice to activation through the T cell receptors.[3] Different changes of the regulation control by the FOXP-3 of FOXP-3-dependent genes result in alteration of Tregs functions and also because of further specific autoimmune disorders, such as systemic lupus erythematosus, rheumatoid arthritis, ulcerative colitis, Crohn's disease, celiac disease, hemolytic anemia, and asthma.[4] All genetic abnormalities that result in the onset of the IPEX syndrome and other selective syndromes as a consequence of mutations in genes dependent on FOXP-3 can be explained mainly by unspecific autoimmune aggression that is attributed to some aspects of the regulation of T cells and also the misbalance of regulation of FOXP-3-dependent genes, which is the result of separate manifestations of IPEX syndrome. In recent reports by Sugimoto et al. (2006),[5] Zheng et al. (2007),[6] and Hill et al. (2007),[7] these authors were the first who indicated the presence of FOXP-3-related genes in natural and induced Tregs by detecting differences in marker genes in CD4$^+$CD25$^-$ and CD4$^+$CD25$^+$ cells.[5—7]

In this article, 10 genes were selected from all FOXP-3-dependent genes detected in the investigations of Sugimoto et al. (2006),[5] Zheng et al. (2007),[6] and Hill et al. (2007)[7] in connection with any of the following criteria: high degree of down- or upregulation of separate gene expression after functional activation of Tregs and high FOXP3 gene expression; proof of synchronous expression of FOXP3 gene and protein and selected gene expression by other authors; and the importance of genes in regulations of immune reactions

and CD4$^+$CD25$^+$ cells functions. The genes described were divided into two groups: one of them is a group of genes capable of changing the immune response and the other group includes genes that may regulate apoptosis and tumorigenesis. The influence [induction ($+$) or inhibition ($-$)] of high expression of FOXP-3 on every described gene expression is indicated in Table 1.

Immune response regulation

The main function of FOXP-3 is to influence Tregs activity and, as a result, there is a possibility to regulate the activity of the immune reactions. The group of FOXP-3-dependent genes that are known to code separate proteins that may change the immune response was indicated. FOXP-3 may change expression of these genes because of suppression or activation of immune response members. The most well-known genes from the aforementioned group include the cytotoxic T-lymphocyte-associated 4 (CTLA-4); inducible costimulator (ICOS); cyclic Adenosine Monophosphate-responsive element modulator (CREM); and PR [positive regulatory domain I—binding factor 1 (PRDI-BNF1)-RIZ] domain containing 1, zinc finger (PRDM1) genes.[5—7]

Cytotoxic T-lymphocyte-associated 4

The CTLA-4 protein is coded by CTLA-4 gene, and it is similar to the lymphocyte CD28 molecule structure, with the exception that it is more competitive than CD28. CTLA-4 has a higher binding affinity to CD80 (B7.1) and CD86 (B7.2) in antigen-presenting cells.[8] Furthermore, inhibition of monocytes and macrophages by CTLA-4 is one of the primary mechanisms to repress the immune response by Tregs.[9] The up-expression of this receptor protein is also regulated by the FOXP-3 protein in natural and induced Tregs.[10] Interestingly, the application of the CTLA-4 antibody or specific deficiency of CTLA-4 caused the expected effects of severe yet reversible T cell-mediated autoimmune disease.[11] The low level of CTLA-4 expression was observed in patients with juvenile idiopathic arthritis.[12] Some human studies had shown that the administration of antibody to CTLA-4 resulted in tumor necrosis.[8]

Inducible costimulator

Another marker of FOXP-3 activity is ICOS.[13] The ICOS gene codes a protein ICOS, which participates in T cell receptor stimulation of T cells. In this case, CD28 is not required for the induction of ICOS. Data have indicated that there is an association between the expression of ICOS and the production of some cytokines. High expression of ICOS was correlated with the production of interleukin (IL)-10 in Tregs.[14] Two subpopulations of CD4$^+$CD25$^+$ natural Tregs were detected. One subset expressed ICOS and another ICOS-negative subpopulation of Tregs. The ICOS$^+$ Tregs produced IL-10 and transforming growth factor (TGF)-β to suppress T cells and dendritic cells. The ICOS$^-$ Tregs produced TGF-β only to suppress T cells. Development of Type 1 diabetes mellitus and common variable immunodeficiency was associated with disturbances of ICOS expression in Tregs.[15]

Table 1 The plus—minus effect of FOXP-3 on some gene expressions in CD4$^+$CD25$^+$ regulatory T cells

Gene	Synonyms	Influence of FOXP-3 on gene expression[a]	References
CTLA-4	CD, CD152, CD28, CELIAC3, CTLA-4, GSE, IDDM12, insulin-dependent diabetes mellitus 12	+	Sugimoto N et al. (2006)[5]; Zheng Y et al. (2007)[6]; Zheng SG et al. (2006)[10]; Hill JA et al. (2007)[7]; Holmberg D et al. (2005)[11]
ICOS	Activation-inducible lymphocyte immunomediatory molecule, AILIM, CD278, inducible T cell costimulator, MGC39850	+	Sugimoto N et al. (2006)[5]; Zheng Y et al. (2007)[6]; Hill JA et al. (2007)[7]; Strauss L et al. (2008)[13]; Honkanen J et al. (2008)[15]
ICER/CREM	cAMP-responsive element modulator, hCREM-2, ICER, inducible cAMP early repressor, MGC111110, MGC17881, MGC41893	+	Sugimoto N et al. (2006)[5]; Zheng Y et al. (2007)[6]; Hill JA et al. (2007)[7]; Bodor J et al. (2007)[16]; Bodor J et al. (2007)[19]
PRDM1	Beta-interferon gene positive regulatory domain I—binding factor, BLIMP1, BLIMP-1, MGC118922, MGC118923, MGC118924, MGC118925, positive regulatory domain I—binding factor 1, PRDI-BF1, PRDI-binding factor 1, PR domain-containing protein 1, PR domain zinc finger protein 1	+	Sugimoto N et al. (2006)[5]; Zheng Y et al. (2007)[6]; Hill JA et al. (2007)[7]; Seddiki N (2009)[21]
GITR	Activation-inducible TNFR family receptor, AITR, GITR, GITR-D, glucocorticoid-induced TNFR-related protein, tumor necrosis factor receptor superfamily member 18, UNQ319/PRO364	+	Sugimoto N et al. (2006)[5]; Zheng Y, et al. (2007)[6]; Hill JA, et al. (2007)[7]; de Boer OJ et al. (2007)[26]
PD-1	CD279, hPD-1, hPD-l, PD1, programmed cell death protein 1, protein PD-1, SLEB2	+	Sugimoto N et al. (2006)[5]; Zheng Y et al. (2007)[6]; Hill JA et al. (2007)[7]; Wang L et al. (2008)[28]
IL-7Rα	CD127, CDw127, CDW127, IL7RA, IL-7R-alpha, ILRA, interleukin-7 receptor subunit alpha	−	Sugimoto N et al. (2006)[5]; Zheng Y et al. (2007)[6]; Hill JA et al. (2007)[7]; Liu W et al. (2006)[32]; Shen LS et al. (2009)[33]
NRP1	BDCA4, CD304, DKFZp686A03134, DKFZp781F1414, neuropilin-1, NP1, NRP, vascular endothelial cell growth factor 165 receptor, VEGF165R	+	Sugimoto N et al. (2006)[5]; Zheng Y et al. (2007)[6]; Hill JA et al. (2007)[7]; Bielenberg DR et al. (2006)[34]; Hansen W et al. (2006)[35]
TGFBR1 and TGFBR2	AAT5, activin receptor-like kinase 5, ACVRLK4, ALK5, ALK-5, LDS1A, LDS2A, serine/threonine-protein kinase receptor R4, SKR4, TbetaR-I, TGF-beta receptor type 1, TGF-beta receptor type I, TGF-beta Type I receptor, TGFR-1, transforming growth factor-beta receptor Type I	+	Sugimoto N et al. (2006)[5]; Zheng Y et al. (2007)[6]; Hill JA et al. (2007)[7]; Li X et al. (2007)[37]
ECM1	Extracellular matrix protein 1, secretory component p85	+	Sugimoto N et al. (2006)[5]; Zheng Y et al. (2007)[6]; Hill JA et al. (2007)[7]; Miyara and Sakaguchi (2007)[39]; Wang L et al. (2003)[40]

[a] "+" Indicates upregulation of gene expression and "−" indicates downregulation of gene expression.

cAMP = cyclic Adenosine Monophosphate; CREM = cAMP-responsive element modulator; CTLA-4 = cytotoxic T-lymphocyte-associated 4; ECM1 = extracellular matrix protein 1; FOXP-3 = forkhead/winged-helix family box protein P3; GITR = glucocorticoid-induced TNFR related; ICER = inducible cAMP early repressor; ICOS = inducible costimulator; IL-7Rα = interleukin-7Rα; NRP-1 = neuropilin-1; PRDI-BF1 = positive regulatory domain I—binding factor 1; PRDM1 = positive regulatory domain I—binding factor 1-RIZ domain containing 1, zinc finger; TGFBR1 = transforming growth factor receptor 1; TGFBR2 = transforming growth factor receptor 2.

cyclic Adenosine Monophosphate-responsive element modulator

CREM is upregulated in Tregs, located in natural and induced Tregs, and is dependent on FOXP-3 expression.[16] CREM protein activity is dependent on the concentration of intracellular calcium, which in turn regulates the activity of calcineurin and nuclear factor of activated T-cells (NFAT). Ca^{2+} signaling leads to the activation of the members of the NFAT in the absence of AP-1. NFAT/activator protein (AP)-1 is a transcriptional complex, which upregulates the expression of genes involved in immune response. In the absence of AP-1, NFAT promotes T cell anergy program. Anergized T cells express increased levels of IL-10.[17] CREM modulates immune response, which was observed in the regulation of antimicrobial defense and immunity.[18] CREM also regulates cyclooxygenase-2, which suppresses effector FOXP3 T cells by a prostaglandin E_2-dependent mechanism.[19] The role of CREM in the pathogenesis of diseases was observed in systemic lupus erythematosus and heart failure.[20]

PR (PRDI-BF1-RIZ) domain containing 1, zinc finger

PRDM1 is another protein-coding FOXP3-related gene that is expressed in Tregs.[21] PRDM1 was originally identified as PRDI-BF1, which functions as an inducible repressor of the transcription of the β-interferon gene expression.[22] This gene is essentially needed for terminal differentiation of B-lymphocytes to antibody-producing plasma cells. It highly expressed in effector and memory T cells of both CD4 and CD8 subpopulations. PR domain zinc finger protein 1 (Beta-interferon gene positive regulatory domain I-binding factor) deficiency in T cells is because of enhanced proliferation and lethal multiorgan inflammatory disorders.[22] Blimp-1 may directly and indirectly repress interleukin 2 gene by means of FBJ murine osteosarcoma viral oncogene homolog, which is one of the components of AP-1 and is a strong activator of interleukin 2 gene. In the absence of Blimp-1, IL-2 levels might greatly increase, which causes autoaggressive response.[23]

Regulation of apoptosis and tumorigenesis

The other primary function of FOXP-3 is the regulation of genes coding apoptotic proteins. FOXP-3 expression induces apoptotic surface factors and suppresses intracellular and nucleic factors, including glucocorticoid-induced tumor necrosis factor receptor (TNFR)-related (GITR) protein, programmed cell death (PD)-1, and IL-7Rα.[5–7] The FOXP-3 protein may regulate genes that are involved in tumorigenesis.[24] Tumor cells release tumor growth factor beta in addition to some other factors that activate the proliferation of Tregs. Antibody-mediated FOXP-3 protein therapy induced apoptosis in cancer cells and inhibited metastasis, which was observed in breast, ovarian, and colon cancer cells.[25] FOXP-3 may be expressed by tumor cells. FOXP-3 also controls genes known to facilitate both the oncogenic transformation of cells and the processes of metastatic progression, such as neuropilin-1 (NRP-1), transforming growth factor beta receptor (TGFBR) 1, TGFBR2, and extracellular matrix protein 1 (ECM1).

Glucocorticoid-induced TNFR-related protein

GITR protein, a receptor belonging to the TNFR superfamily, is activated by GITR ligand (GITRL). GITRL belongs to the tumor necrosis factor family and is a Type II transmembrane protein. GITRL is expressed on antigen-presenting cells and B cells but not in T cells. GITR is expressed at low levels in resting responder T cells and is highly expressed in Tregs, and it is recognized as a marker of Tregs.[26] GITR protein stimulates T cell–mediated anti-tumor immunity in mice. In humans, high expression of GITR in primary gastrointestinal cancers and tumor cell lines of different histological origin was observed.[27]

PD-1

The PD-1 belongs to CD28 family of receptors, expressed on T cells, B cells, and myeloid cells. These receptors are transmembrane proteins and are expressed as monomers. PD-1 binds to PD-L1 (B7-H1) and PD-L2 (B7-DC), which are homologs of B7-1 and B7-2 and are induced on antigen-presenting cells, and on various nonimmune cells. PD-1 negatively regulates cell activation and proliferation, causing cell cycle arrest without apoptosis. PD-1 recruits the protein tyrosine phosphatase, non-receptor type 11. PD-1 inhibits chronically activated cells and responses in peripheral, nonlymphoid tissues. PD-1 is expressed in Tregs, which promote the regulation of immune responses by these cells.[28] PD-1 may have intra- and extracellular localization. Activation of Tregs with high expression of FOXP-3 results in extracellular expression of PD-1.[29] Mice with knockout PD-1 gene develop autoimmune cardiomyopathy or a lupus-like syndrome. In humans, part of the immune deficiency associated with HIV infection may also be because of PD-1-mediated inhibition of T cell activation.[30]

Interleukin-7Rα

The IL-7Rα (CD127) is a Type I cytokine receptor chain devoid of intrinsic tyrosine kinase activity. It can bind IL-7 because of janus kinase 3-mediated phosphorylation of IL-7Rα and recruitment of janus kinase 2 and signal transducer and activator of transcription 5 transcription factors. IL-7 stimulates survival and expansion of immature precursors committed to the T and B cells. Patients with IL-7Rα deficiency present with signs and symptoms of severe combined immune deficiency, characterized by failure to thrive, recurrent otitis, viral infections, candidiasis, diarrhea, and fever.[31] Although most $CD4^+$ human T cells express IL-7Rα, Tregs exhibiting suppressive activity *in vitro* display distinctly lower surface expression of this marker. Expression of CD127 negatively correlates with FOXP-3 content, because FOXP-3 interacts with the CD127 promoter, contributing to the low expression of CD127 in $CD4^+CD25^+$ Tregs.[32] Recent publications also propose that high expression of CD127 can modulate Tregs functions and decrease its suppressive activity. This fact was observed in patients with gastric cancer.[33]

Neuropilin-1

NRP1 codes NRP-1 protein. NRP-1 is a surface marker of $CD4^+CD25^+$ Tregs. NRPs are membranous receptors capable of binding two disparate ligands, Class 3 semaphorins, and vascular growth factor, and regulating two diverse systems, neuronal guidance, and angiogenesis. NRP-1 is predominantly expressed on carcinomas.[34] The expression of NRP-1 by tumor cells is of great interest because NRP-1 and TGF-β1 have been linked to cancer progression. NRP-1 is an important factor that binds to TGF-β1 in its activated and latency-associated peptide forms. NRP-1 is a receptor for TGF-β1 in Tregs, and it promotes Tregs activity.[35] In recent publications, it was proposed to use NRP-1 blockers for treatment of cancers.[36]

TGFBR1 and TGFBR2

Another FOXP3-related gene is TGFBR1. This gene codes protein TGF-βR1, which interacts with TGF-βR2 for further formation of complex with TGF-β1. Biochemically, TGF-βR1 and TGF-βR2 are serine/threonine kinases, which phosphorylate Smads. Furthermore to Smads, TGF-β1 also activates other signaling pathways, including various mitogen-activated protein kinases and phosphatidylinositol-3 kinase. TGF-β1 downregulates the proliferation and effector functions of T cells and the activation of macrophages. TGF-β and TGF-βR1 regulates differentiation or survival of Tregs. TGF-β signaling induces expression of the FOXP-3 and generation of Tregs from activated $CD4^+$ T cells.[37] TGF-β signaling is frequently perturbed in many human cancers, including renal cell carcinomas, colorectal cancer, non-small-cell lung cancer, and transitional cell carcinoma.[38]

Extracellular matrix protein 1

The ECM1 gene codes similarly named extracellular matrix protein. This is a secreted protein that has been implicated with extracellular matrix formation, cell adhesion, cell signaling, and regulation of tissue differentiation and maturation. Mutation of the ECM1 gene, leading to autosomal-recessive genodermatosis, lipoid proteinosis, also known as Urbach—Wiethe disease, which is characterized by generalized thickening of the skin and mucosal infiltration with scarring. ECM-1 interacts with other extracellular matrix components, such as polysaccharides (hyaluronan, heparin, chondroitin sulfate A) and proteins [fibronectin, collagen IV, matrix metallopeptidase (MMP-9)], either as its reservoir or as its cofactor. The ECM-1 protein supports the processes of angiogenesis. It stimulates the proliferation of endothelial cells and promotes blood vessel formation, which may be the result of tumor progression and metastasis formation. Expression of ECM-1 closely correlates with the expression of FOXP-3 in $CD4^+CD25^+$ Tregs.[39] High expression of ECM-1 factor indicates the poor prognosis and metastatic progression of invasive breast ductal carcinoma, esophageal squamous carcinoma, gastric cancer, and colorectal cancer.[40]

The primary function of FOXP-3-dependent genes is regulation of the immune responses by means of changes of $CD4^+CD25^+$ Tregs functionality, which may lead to disorders of the metabolism of various substances, disruptions in hormonal regulation, and misbalance in the cell cycle, and others. In this review, the main (immune response, apoptosis, and tumorigenesis regulation) functions of 10 FOXP-3-dependent genes are described, although many of FOXP-3-dependent genes and their functions remain unknown. Understanding the biological properties of FOXP-3 would have a wide range of implications for salvation of problems of immune escape in cancers, treatment of autoimmune disorders, regulation of inflammation, and immune response to infectious diseases. It is possible that the development of new strategies with genetic manipulations with the aim to change the expression of FOXP3 or FOXP-3-dependent genes may be helpful in the elaboration of new principles for treatment of many diseases and theirs complications.

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