REVIEW

Foxp3 expression in T regulatory cells and other cell lineages

Christel Devaud · Phillip K. Darcy ·
Michael H. Kershaw

Abstract Forkhead box P3 (Foxp3) is an important transcription factor that belongs to the forkhead/winged-helix family of transcriptional regulators. Foxp3 has been extensively studied over the past 13 years as a master regulator of transcription in a specific T-cell type, $CD4^+$ regulatory T cells (Treg), both in humans and in mice. Compelling data characterize Foxp3 as critically important and necessary for the development and the differentiation of Treg. It has been considered initially as the only specific marker for Treg. However, recent work has proposed that Foxp3 can be expressed by other types of lymphoid cells or myeloid cells and also by some non-hematopoietic cells such as epithelial cells. It remains controversial about the expression of Foxp3 in cells other than Treg, but understanding the potential expression and function of this master regulator in different cell subsets could have a wide range of implications for immune tolerance and several pathologies including autoimmune disorders and immune responses to cancer.

Abbreviations

BM	Bone marrow
BRCA1	Breast cancer 1, early onset
CD	Cluster of differentiation
CTLA4	Cytotoxic T-lymphocyte antigen-4
DC	Dendritic cells
DEREG	Depletion of regulatory T cells
DNA	Deoxyribonucleic acid
DT	Diphtheria toxin
EAE	Experimental autoimmune encephalomyelitis
FOX	Forkhead box
GITR	Glucocorticoid-induced tumor necrosis factor receptor family related gene
HAT	Histone/protein acetyltransferases
HER	Human epidermal growth factor receptor
IL	Interleukin
IL2R	Interleukin 2 receptor
NK	Natural killer
iNKT	Invariant natural killer T cells
IPEX	Immune dysregulation, polyendocrinopathy, entheropathy, X-linked
IRF	Interferon regulatory factor
PBMC	Peripheral blood mononuclear cells
Rag	Recombination-activating genes
RNA	Ribonucleic acid
ROR	Retinoid-related orphan receptor
SATB	Special AT-rich sequence binding protein
SKP	S-phase kinase-associated protein
TGF-β	Transforming growth factor beta
Treg	T-regulatory cells
WT	Wild type

C. Devaud · P. K. Darcy · M. H. Kershaw
Cancer Immunology Research Program, Sir Peter MacCallum
Department of Oncology, University of Melbourne, Parkville,
VIC 3010, Australia

C. Devaud (✉) · M. H. Kershaw (✉)
Cancer Immunology Research Program, Peter MacCallum
Cancer Center, Saint Andrews Place, Melbourne,
VIC 3002, Australia
e-mail: christel.devaud@petermac.org

M. H. Kershaw
e-mail: michael.kershaw@petermac.org

P. K. Darcy · M. H. Kershaw
Department of Immunology, Monash University, Prahran,
VIC 3181, Australia

Introduction

Forkhead box P3 (Foxp3) is a transcription factor encoded on the X chromosome that belongs to the family of forkhead box (FOX) transcription factors characterized by a highly conserved forkhead DNA-binding domain. Similar to other members of the family, Foxp3 also has a leucine zipper-like domain and zinc finger motif. The N terminus domain is thought to be the repressor domain [1]. The molecule is expressed as one isoform full-length protein in mice, while two major isoforms were identified in humans [2]. Foxp3 is generally part of a large molecular complex of around 600 kDa [3, 4] and can interact with many other transcription factors such as IRF4 and ROR γ [5, 6].

The *Foxp3* gene was originally characterized during studies of mouse and human IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) in which *Foxp3* was found to be mutated [7–10]. Indeed, mice and humans affected with a loss-of-function mutation in the *Foxp3* gene were afflicted with a fatal, early-onset, autoimmune disorder. Early studies in the mouse mutant strain scurfy revealed an essential role of T cells in the observed pathologies [11, 12]. Initially, the Foxp3 transcription factor was clearly demonstrated to be specifically expressed by some immunosuppressive CD4$^+$ T cells constitutively expressing the α-subunit of the interleukin (IL)-2 receptor (CD25) on their surface [13–15]. Subsequently, descriptions of its expression have been extended to other cell types, including non-hematopoietic cell types such as normal and malignant tumoral cells.

However, the immunosuppressive nature of Foxp3-expressing Treg can be manifested in discrepant roles in cancer. On the one hand, they may suppress the effector immune response and help the tumor grow, while on the other hand, they may downregulate inflammation, thereby protecting the host from tumor progression. Treg are known to promote some cancers, including Hodgkin lymphoma, melanoma, breast, gastric and ovarian carcinoma. However, in other malignancies, including head and neck cancer, colorectal and bladder cancer, Treg infiltration correlates with a better control of tumors in patients [16]. This dual role of Treg has been recently reviewed and illustrates the complexity of this T-cell subset [17].

In this review, we will not summarize literature about Foxp3 function or biology in Treg that has been largely covered [18–20], but we will summarize the latest literature on the expression of Foxp3 protein in different cell types. The latest controversy on potential Foxp3 expression in macrophages will also be discussed.

Foxp3 in T cells

In 1995, Sakaguchi et al. [21] highlighted a subset of CD4$^+$ T cells, expressing CD25 that exhibits a potent suppressor activity. Further work from this author and his group revealed that these cells, termed Treg, displayed a high level of CD5 and cytotoxic T-lymphocyte antigen-4 (CTLA-4) [22]. Later, they showed that Treg express high levels of Foxp3 mRNA and protein and also that forced expression of Foxp3 in CD4$^+$CD25$^-$ T cells resulted in acquisition of suppressor function and a Treg cell phenotype. This result prompted the idea that Foxp3 was essential for immunosuppressive Treg differentiation [13–15]. Further studies demonstrated that the amount of Foxp3 protein in Tregs was critical for their suppressive function [23] and to a large extent stabilizes their molecular features. Therefore, Foxp3 has been considered to be a lineage-specific transcription factor of CD4$^+$CD25$^+$ Treg cells specialized in the negative regulation of the immune response [24]. Treg are known to be particularly abundant in tumor tissues in particular. Indeed, they can be found in large numbers in tumors, including cancers of various origin such as breast, lung, liver, pancreatic and gastrointestinal cancer and malignant melanoma [25]. Poor prognosis in ovarian, breast and gastric cancer has been associated with the presence of a large proportions of CD4$^+$Foxp3$^+$Treg among tumor-infiltrating lymphocytes [26] and a decreased ratio of CD8$^+$ effector T cells to Foxp3$^+$Treg cells [27]. These findings suggested that tumor-reactive CD8$^+$ cytotoxic T cells are suppressed by Foxp3$^+$ Treg in tumor tissues. Furthermore, Treg can suppress the antitumor functions of many other immune cell subsets in tumors, including natural killer (NK) cells, NK T cells, B cells, macrophages and dendritic cells (DC) [28]. By exerting three major functional mechanisms, including production of immunosuppressive factors, suppression by direct cell–cell contact and cytolysis, Treg can impact on the tumor growth. Consequently, depletion of specific Foxp3-expressing Treg or attenuation of their suppressive functions can restore antitumor immunity and trigger the elimination of tumors [29].

However, there are several indications that Foxp3 expression per se might not be restricted to immunosuppressive Treg. For example, conventional activated human CD4$^+$ T cells could transiently express Foxp3 at a low level but did not exhibit suppressive activity [30]. Furthermore, a cell population among human blood circulating CD4$^+$ T cells was found to express Foxp3, but not exhibit suppressive activity, and was also able to produce pro-inflammatory cytokines upon activation [31]. Taken together, these findings combined with observations of others [32] suggested that the concept of Treg development and function determined by Foxp3 is not as simple as widely accepted. Furthermore, expression of the Foxp3 transcription factor might not be restricted to only CD4$^+$ Treg cells.

Some CD8$^+$ T-cell subsets, commonly termed CD8$^+$ regulatory T cells, have been demonstrated to express Foxp3. These CD8$^+$ Treg cells expressing Foxp3 have been shown

to be induced in vitro through TCR-dependent stimulation and to exhibit immunosuppressive activity. The induction of some CD8$^+$CD25$^+$Foxp3$^+$ subsets was observed in vitro following continuous antigen stimulation in the presence of CD14$^+$ monocytes [33] or both in vitro and in vivo following stimulation with a genetically modified anti-CD3 monoclonal antibodies [34]. In addition, some autoreactive CD8$^+$CD25$^+$CTLA-4$^+$Foxp3$^+$ T-cell clones have been isolated from healthy individuals or ankylosing spondylitis patients using autologous LPS-activated dendritic cells [35]. Other studies demonstrated that CD8$^+$Foxp3$^+$ T cells could be derived from human PBMC through in vitro stimulation using hepatitis C- or flu virus-specific peptides [36] and bacillus Calmette–Guérin antigen [37]. Some natural CD8$^+$ Treg expressing Foxp3 could constitute an endogenous long-lived population of T cells. For example, some CD8$^+$CD25$^+$CTLA-4$^+$GITR$^+$Foxp3$^+$ T cells share functional and phenotypic features with CD4$^+$CD25$^+$ regulatory T cells [38]. Several studies reported a strong suppressive activity of CD8$^+$Foxp3$^+$ Treg on CD4 and CD8 conventional T-cell proliferation [33, 34]. However, Foxp3 does not seem to be necessary for the immunosuppressive function of CD8$^+$ Treg, as the murine CD8$^+$CD122$^+$ and CD8$\alpha\alpha^+$ Treg populations are Foxp3-negative [39, 40]. In addition, Mayer et al. [41] recently demonstrated that CD8$^+$Foxp3$^+$ T cells share some phenotypic characteristics with CD4$^+$ Treg but lack potent suppressive activity.

Interestingly, some studies recently proposed that invariant NKT (iNKT) cells could potentially express Foxp3. Monteiro et al. [42] identified a population of iNKT cells expressing Foxp3 in the context of experimental autoimmune encephalomyelitis in mice. It was then further confirmed, in both mice and humans, that Foxp3 could be expressed on unstimulated iNKT cells or induced through exposure with TGF-β [43].

Foxp3 in normal and malignant epithelial cells

Some data indicate that Foxp3 could be expressed by some non-lymphoid cells, in particular epithelial cells. For example, Chang et al. proposed that Foxp3 plays a role in the regulation of double negative thymocyte maturation and detected its expression in the thymic epithelium [44]. However, this work remains controversial as another study found no evidence for Foxp3 expression or function in the thymic epithelium [45].

Forkhead box P3 could potentially act as a tumor suppressor gene in some cancers. This was reported to be the case in breast and prostate cancer in particular, where Foxp3 expression was found in normal epithelial cells and its downregulation was related to cancer development [46, 47]. Indeed, Foxp3 mRNA and protein have been detected in the nuclei of mouse epithelial cells in breast, lung and prostate

in Rag2−/− and scurfy mice [46]. It has also been detected in human breast and prostate epithelium [47]. Interestingly, it was proposed that Foxp3 could repress expression of some oncogenes involved in mammary carcinogenesis and breast tumor growth and metastasis, including *HER2*, *SKP2* [48] and *SATB1* [49]. Furthermore, in prostate cells, Foxp3 has been shown to directly repress *c-myc* transcription, an oncogene frequently overexpressed in many human cancers [50]. More recently, a study demonstrated using different human cell lines that Foxp3 was upregulated following γ-irradiation and able to suppress the expression of the DNA-repair tumor suppressor gene *BRCA1* [51].

In contrast, some groups have proposed that Foxp3 is involved in the biology of cancer as they demonstrated that the protein was expressed more highly in tumor cells than in corresponding epithelial cells. Analyses done on large series of human breast cancer specimens demonstrated Foxp3 expression in breast carcinoma [52, 53]. Hinz et al. found no Foxp3 expression in normal pancreatic duct cells, while it was detected using immunohistochemistry in human pancreatic cancer cells. In that study, the authors proposed that Foxp3 could be used as a mechanism of immune evasion for the cancer cells [54]. Other investigators found Foxp3 expressed by human melanoma cells from fresh tissue and suggested that malignant transformation of healthy cells could induce Foxp3 expression [55]. More recently, Foxp3 protein expression was detected by immunohistochemistry and Western blot in human parenchymal cells from cervical esophageal cancer [56], gastric tumor cells [57, 58] and invasive ductal breast carcinoma [59]. Furthermore, expression of Foxp3 mRNA has been observed in several human cancer cell lines [60] and recently in the mouse B16F10 melanoma cell line [61].

It is important to note that most of those studies are controversial given that other studies have found no evidence of Foxp3 expression in non-lymphoid cells using genetic, cellular and immunohistochemical approaches [62, 63]. Recently, in a large study performed on human breast carcinoma samples and cell lines, combining multiple techniques of analysis, the authors were able to detect Foxp3 expression in <1 % of breast cancer. They proposed that Foxp3 does not play a role in breast cancer biology [64]. In contrast, another study suggests that Foxp3 expression in human melanoma cells can provide a Treg-like activity to the tumor cells, rendering them able to suppress T-cell proliferation. That study proposed Foxp3 as a possible mechanism of tumor resistance to the immune system in the melanoma context [65].

Foxp3 in myeloid cells

In a retracted study [66], Zorro-Manrique et al. claimed that a population of mouse CD11b$^+$F4/80$^+$CD68$^+$ macrophages

expressed Foxp3 and possessed some immunosuppressive functions able to promote tumor growth. This study opened a new debate on the potential expression of Foxp3 in myeloid cells; however, there have been no other reports demonstrating expression of Foxp3 in macrophage subsets.

Three papers and comments published in 2012 described the absence of expression of Foxp3 in macrophages. Put et al. [67] reported in *Blood* that they were not able to detect Foxp3 mRNA or protein in macrophages derived from naïve bone marrow (BM) and spleen or in the pathological context of collagen-induced arthritis and also under GM-CSF/IL4 activation conditions in vitro. However, in this study, analyses were performed only in C57BL/6 mice and not extended to tissues other than BM and spleen or to further pathological models such as cancer. It remains possible that Foxp3 could be expressed in macrophages from different organs and in different disease contexts. In the same issue of *Blood*, Mayer et al. [68] presented further evidence of no Foxp3 expression in CD11b$^+$ cells from WT or DEREG naive and B16 melanoma-bearing mice spleen and BM. In the Mayer et al. study, the authors used an anti-Foxp3 antibody and a CD11b$^+$Foxp3^{low} population was identified. Mice expressed the diphtheria toxin receptor under the control of the Foxp3 promoter in this study, but the Foxp3^{low} population persisted after diphtheria toxin (DT) administration. However, a potential low level of Foxp3 in the cells implied a low level of DT receptor on the surface of the cells, which may not have been sufficient to deplete these cells using DT. Finally, a recent paper from Li et al. [69] that employed flow cytometry suggested that false Foxp3 positive staining macrophages could be observed due to autofluorescence of the cells but no positive control (on TReg) supporting the effectiveness of their intranuclear staining was included in the report.

In some of our recent unpublished works (Devaud et al. unpublished data, March 2014), we could detect the expression of the Foxp3 transcription factor in some F4/80$^+$/CD11b$^+$ macrophages. We were able to demonstrate the expression of Foxp3 at the protein level using flow cytometric analysis and Western blot analysis, particularly in type2 macrophages infiltrating orthotopic renal tumors that we described in a previous study [70]. We found that the identified Foxp3 protein was at a larger size (around 65 kDa) than the expected size (52 kDa). Characterization of this protein awaits some analysis through mass spectroscopy. Furthermore, we also detected a Foxp3 mRNA transcript variant that was previously unknown and not present in some CD4$^+$ T cells. Extended studies need to be performed to describe in detail the Foxp3 protein and new mRNA variant identified in macrophages and also understand the potential immunoregulatory function in these cells.

Targeting Foxp3 in cancer immunotherapy

Usually considered as a key immunoregulatory subset in tumors, Foxp3-expressing Treg have often been targeted in order to enhance anticancer immunotherapy. Several strategies, neutralizing Treg, have been proposed in order to directly induce the elimination of tumors or improve current immunotherapies. Indeed, low doses of chemotherapy administration [71] or targeting the α-subunit of the IL2R, using a fusion protein of IL-2 and diphtheria toxin [72] or anti-CD25 depleting antibodies [73], have shown high efficacy in depleting Treg and can augment antitumor immunotherapy efficacy. However, all these approaches lack specificity for immunosuppressive Treg and can also eliminate effector antitumor T cells. Furthermore, the depletion of Treg using those strategies could lead to autoimmunity.

An alternative way to control Treg in the tumor microenvironment is possible through the direct inhibition of Foxp3-related functions. Indeed, as Foxp3 is crucial to maintain Treg phenotype and function, its ablation results in the loss of suppressive potential [74]. Considering the difficulties to target Foxp3, due to its intracellular location, one strategy proposed to use cell-penetrating small peptide inhibitors. Recently, a 15-mer synthetic peptide (called P60) was identified as able to bind Foxp3 protein and prevent its nuclear translocation in Treg. Consequently, a P60 treatment combined with a tumor-associated epitope immunization protected mice against tumor implantation by reducing the Treg immunosuppressive potential [75]. Another arginine-rich, antisense peptide-conjugated phosphorodiamidate morpholino, structurally similar to RNA, can be taken up, in vitro, by activated T cells and trigger a downregulation of Foxp3 expression in the T cells [76]. Foxp3 has also been targeted in vaccination technology that uses Foxp3 RNA-transfected DC and elicits a robust Foxp3-specific cytotoxic T-cell response. Importantly, this type of vaccination depletes specifically the intratumoral but not the peripheral Treg, opening an interesting perspective to avoid autoimmunity following Treg depletion [77]. Finally, the disruption of specific elements of the Foxp3 interactome can be considered using, for example, small molecule targeting histone/protein acetyltransferases (HAT) [78]. Indeed, conditional deletion or pharmacologic inhibition of p300 HAT can specifically impair Treg suppressive functions and limit tumor growth in immunodeficient mice [79].

Designing strategies directly targeting Foxp3 are initially considered to inhibit Treg, yet these strategies might extend the possibilities of targeting Foxp3-expressing tumor cells or immunosuppressive myeloid cells in future therapies. However, one has to consider the potential beneficial effect of Foxp3 on epithelial cells, as a tumor suppressor gene. Interestingly, in a study described previously,

Niu et al. found that the downregulation of Foxp3 in human melanoma cells, using small-RNA interference, reduces the suppressive abilities of the cells by downregulating B7-H1 and TGF-β expression [65]. More recently, the same group used the vaccine strategy with human Foxp3 RNA-transfected DC, in order to stimulate T cells to lyse inflammatory breast cancer cells [80].

Concluding remarks

Much research into inflammatory diseases, autoimmunity and cancers in particular, underlines the importance of CD4$^+$ Treg cells in the control of the immune response. Understanding Treg suppressive functions and how they rely on the specific transcription factor Foxp3 was incredibly helpful in the design of more effective therapies [81]. However, an extended analysis of the current literature on Foxp3 clearly presents the possibility that Foxp3 transcription factor expression may not be restricted to CD4$^+$ T regulatory cells.

We have highlighted in this review how some other T cells CD4$^+$ or CD8$^+$ could express Foxp3. However, surprisingly, in those cells, Foxp3 expression does not automatically mean consequent immunosuppressive functions [31, 41]. This implies other unexplored functions of Foxp3 in these cells.

In addition to the transcription regulatory role on immune genes, other potential roles for Foxp3 are illustrated by recent studies proposing Foxp3 expression in epithelial normal and tumoral cells. Indeed, Foxp3 seems to have been recently considered as either a suppressor gene or an oncogene in cancer biology [82]. One could argue that the controversy existing in this new field of research (Foxp3 expression in tumor cells) illustrates the complexity of this transcription factor and of cancer cells themselves. Particular care should be taken in the analysis of data (in particular, flow cytometric analysis and immunohistochemistry analysis, well represented on the papers detecting Foxp3 in epithelial/tumoral cells) on cancer cells as they are such plastic cells, potentially able to express a full range of markers covering all cell subsets.

Finally, the potential expression of Foxp3 has been extensively criticized after the retraction of a "proof of concept" paper from *JEM* journal [66]. The retraction of the paper followed by two rigorous reports in *Blood* questioned the credibility of a possible expression of Foxp3 by macrophages. Nevertheless, it remains that a retracted study does not automatically negate the entire data set presented in the manuscript. Furthermore, our results showed, using the current available tools to study Foxp3, that Foxp3 expression could be detected in macrophages, suggesting that many unanswered questions remain regarding this transcription factor.

Understanding potential functions of Foxp3 in cells such as macrophages, normal epithelial and tumor cells may open new doors into improving therapies against different pathologies.

Conflict of interest The authors declare that they have no conflicts of interest.

References

1. Li B, Greene MI (2007) FOXP3 actively represses transcription by recruiting the HAT/HDAC complex. Cell Cycle 6(12):1432–1436
2. Allan SE, Passerini L, Bacchetta R, Crellin N, Dai M, Orban PC, Ziegler SF, Roncarolo MG, Levings MK (2005) The role of 2 FOXP3 isoforms in the generation of human CD4+ Tregs. J Clin Invest 115(11):3276–3284
3. Li B, Samanta A, Song X, Iacono KT, Bembas K, Tao R, Basu S, Riley JL, Hancock WW, Shen Y, Saouaf SJ, Greene MI (2007) FOXP3 interactions with histone acetyltransferase and class II histone deacetylases are required for repression. Proc Natl Acad Sci USA 104(11):4571–4576
4. Ono M, Yaguchi H, Ohkura N, Kitabayashi I, Nagamura Y, Nomura T, Miyachi Y, Tsukada T, Sakaguchi S (2007) Foxp3 controls regulatory T-cell function by interacting with AML1/Runx1. Nature 446(7136):685–689
5. Zhou L, Lopes JE, Chong MM, Ivanov II, Min R, Victora GD, Shen Y, Du J, Rubtsov YP, Rudensky AY, Ziegler SF, Littman DR (2008) TGF-beta-induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing RORgammat function. Nature 453(7192):236–240
6. Zheng Y, Chaudhry A, Kas A, deRoos P, Kim JM, Chu TT, Corcoran L, Treuting P, Klein U, Rudensky AY (2009) Regulatory T-cell suppressor program co-opts transcription factor IRF4 to control T(H)2 responses. Nature 458(7236):351–356
7. Brunkow ME, Jeffery EW, Hjerrild KA, Paeper B, Clark LB, Yasayko SA, Wilkinson JE, Galas D, Ziegler SF, Ramsdell F (2001) Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. Nat Genet 27(1):68–73
8. Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L, Kelly TE, Saulsbury FT, Chance PF, Ochs HD (2001) The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nat Genet 27(1):20–21
9. Wildin RS, Ramsdell F, Peake J, Faravelli F, Casanova JL, Buist N, Levy-Lahad E, Mazzella M, Goulet O, Perroni L, Bricarelli FD, Byrne G, McEuen M, Proll S, Appleby M, Brunkow ME (2001) X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. Nat Genet 27(1):18–20
10. Chatila TA, Blaeser F, Ho N, Lederman HM, Voulgaropoulos C, Helms C, Bowcock AM (2000) JM2, encoding a fork head-related protein, is mutated in X-linked autoimmunity-allergic disregulation syndrome. J Clin Invest 106(12):R75–R81
11. Blair PJ, Bultman SJ, Haas JC, Rouse BT, Wilkinson JE, Godfrey VL (1994) CD4+ CD8− T cells are the effector cells in disease pathogenesis in the scurfy (sf) mouse. J Immunol 153(8):3764–3774

12. Godfrey VL, Wilkinson JE, Rinchik EM, Russell LB (1991) Fatal lymphoreticular disease in the scurfy (sf) mouse requires T cells that mature in a sf thymic environment: potential model for thymic education. Proc Natl Acad Sci USA 88(13):5528–5532

13. Schubert LA, Jeffery E, Zhang Y, Ramsdell F, Ziegler SF (2001) Scurfin (FOXP3) acts as a repressor of transcription and regulates T cell activation. J Biol Chem 276(40):37672–37679

14. Fontenot JD, Gavin MA, Rudensky AY (2003) Foxp3 programs the development and function of CD4+ CD25+ regulatory T cells. Nat Immunol 4(4):330–336

15. Hori S, Nomura T, Sakaguchi S (2003) Control of regulatory T cell development by the transcription factor Foxp3. Science 299(5609):1057–1061

16. Tanchot C, Terme M, Pere H, Tran T, Benhamouda N, Strioga M, Banissi C, Galluzzi L, Kroemer G, Tartour E (2013) Tumor-infiltrating regulatory T cells: phenotype, role, mechanism of expansion in situ and clinical significance. Cancer Microenviron 6(2):147–157

17. Whiteside TL (2014) Regulatory T cell subsets in human cancer: are they regulating for or against tumor progression? Cancer Immunol Immunother 63(1):67–72

18. Josefowicz SZ, Lu L-F, Rudensky AY (2012) Regulatory T cells: mechanisms of differentiation and function. Annu Rev Immunol 30(1):531–564

19. Rudensky AY (2011) Regulatory T cells and Foxp3. Immunol Rev 241(1):260–268

20. Sakaguchi S, Miyara M, Costantino CM, Hafler DA (2010) FOXP3+ regulatory T cells in the human immune system. Nat Rev Immunol 10(7):490–500

21. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M (1995) Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol 155(3):1151–1164

22. Takahashi T, Tagami T, Yamazaki S, Uede T, Shimizu J, Sakaguchi N, Mak TW, Sakaguchi S (2000) Immunologic self-tolerance maintained by CD25(+)CD4(+) regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. J Exp Med 192(2):303–310

23. Wan YY, Flavell RA (2007) Regulatory T-cell functions are subverted and converted owing to attenuated Foxp3 expression. Nature 445(7129):766–770

24. Campbell DJ, Koch MA (2011) Phenotypical and functional specialization of FOXP3+ regulatory T cells. Nat Rev Immunol 11(2):119–130

25. Nishikawa H, Sakaguchi S (2010) Regulatory T cells in tumor immunity. Int J Cancer 127(4):759–767

26. Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, Evdemon-Hogan M, Conejo-Garcia JR, Zhang L, Burow M, Zhu Y, Wei S, Kryczek I, Daniel B, Gordon A, Myers L, Lackner A, Disis ML, Knutson KL, Chen L, Zou W (2004) Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat Med 10(9):942–949

27. Sato E, Olson SH, Ahn J, Bundy B, Nishikawa H, Qian F, Jungbluth AA, Frosina D, Gnjatic S, Ambrosone C, Kepner J, Odunsi T, Ritter G, Lele S, Chen YT, Ohtani H, Old LJ, Odunsi K (2005) Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. Proc Natl Acad Sci USA 102(51):18538–18543

28. Sakaguchi S, Yamaguchi T, Nomura T, Ono M (2008) Regulatory T cells and immune tolerance. Cell 133(5):775–787

29. Teng MW, Ritchie DS, Neeson P, Smyth MJ (2011) Biology and clinical observations of regulatory T cells in cancer immunology. Curr Top Microbiol Immunol 344:61–95

30. Allan SE, Crome SQ, Crellin NK, Passerini L, Steiner TS, Bacchetta R, Roncarolo MG, Levings MK (2007) Activation-induced FOXP3 in human T effector cells does not suppress proliferation or cytokine production. Int Immunol 19(4):345–354

31. Miyara M, Yoshioka Y, Kitoh A, Shima T, Wing K, Niwa A, Parizot C, Taflin C, Heike T, Valeyre D, Mathian A, Nakahata T, Yamaguchi T, Nomura T, Ono M, Amoura Z, Gorochov G, Sakaguchi S (2009) Functional delineation and differentiation dynamics of human CD4+ T cells expressing the FoxP3 transcription factor. Immunity 30(6):899–911

32. Ohkura N, Kitagawa Y, Sakaguchi S (2013) Development and maintenance of regulatory T cells. Immunity 38(3):414–423

33. Mahic M, Henjum K, Yaqub S, Bjørnbeth BA, Torgersen KM, Taskén K, Aandahl EM (2008) Generation of highly suppressive adaptive CD8+CD25+FOXP3+ regulatory T cells by continuous antigen stimulation. Eur J Immunol 38(3):640–646

34. Bisikirska B, Colgan J, Luban J, Bluestone JA, Herold KC (2005) TCR stimulation with modified anti-CD3 mAb expands CD8+ T cell population and induces CD8+CD25+ Tregs. J Clin Invest 115(10):2904–2913

35. Jarvis LB, Matyszak MK, Duggleby RC, Goodall JC, Hall FC, Gaston JSH (2005) Autoreactive human peripheral blood CD8+ T cells with a regulatory phenotype and function. Eur J Immunol 35(10):2896–2908

36. Billerbeck E, Blum HE, Thimme R (2007) Parallel expansion of human virus-specific FoxP3−effector memory and De novo-generated FoxP3+ regulatory CD8+ T Cells upon antigen recognition in vitro. J Immunol 179(2):1039–1048

37. Joosten SA, van Meijgaarden KE, Savage NDL, de Boer T, Triebel F, van der Wal A, de Heer E, Klein MR, Geluk A, Ottenhoff THM (2007) Identification of a human CD8+ regulatory T cell subset that mediates suppression through the chemokine CC chemokine ligand 4. Proc Natl Acad Sci 104(19):8029–8034

38. Cosmi L, Liotta F, Lazzeri E, Francalanci M, Angeli R, Mazzinghi B, Santarlasci V, Manetti R, Vanini V, Romagnani P, Maggi E, Romagnani S, Annunziato F (2003) Human CD8+CD25+ thymocytes share phenotypic and functional features with CD4+CD25+ regulatory thymocytes. Blood 102(12):4107–4114

39. Rifa'i M, Kawamoto Y, Nakashima I, Suzuki H (2004) Essential roles of CD8+CD122+ regulatory T cells in the maintenance of T cell homeostasis. J Exp Med 200(9):1123–1134

40. Holler PD, Yamagata T, Jiang W, Feuerer M, Benoist C, Mathis D (2007) The same genomic region conditions clonal deletion and clonal deviation to the CD8alphaalpha and regulatory T cell lineages in NOD versus C57BL/6 mice. Proc Natl Acad Sci USA 104(17):7187–7192

41. Mayer CT, Floess S, Baru AM, Lahl K, Huehn J, Sparwasser T (2011) CD8+Foxp3+ T cells share developmental and phenotypic features with classical CD4+Foxp3+ regulatory T cells but lack potent suppressive activity. Eur J Immunol 41(3):716–725

42. Monteiro M, Almeida CF, Caridade M, Ribot JC, Duarte J, Agua-Doce A, Wollenberg I, Silva-Santos B, Graca L (2010) Identification of regulatory Foxp3+ invariant NKT cells induced by TGF-β. J Immunol 185(4):2157–2163

43. Moreira-Teixeira L, Resende M, Devergne O, Herbeuval JP, Hermine O, Schneider E, Dy M, Cordeiro-da-Silva A, Leite-de-Moraes MC (2012) Rapamycin combined with TGF-beta converts human invariant NKT cells into suppressive Foxp3+ regulatory cells. J Immunol 188(2):624–631

44. Chang X, Gao JX, Jiang Q, Wen J, Seifers N, Su L, Godfrey VL, Zuo T, Zheng P, Liu Y (2005) The scurfy mutation of FoxP3 in the thymus stroma leads to defective thymopoiesis. J Exp Med 202(8):1141–1151

45. Liston A, Farr AG, Chen Z, Benoist C, Mathis D, Manley NR, Rudensky AY (2007) Lack of Foxp3 function and expression in the thymic epithelium. J Exp Med 204(3):475–480

46. Chen GY, Chen C, Wang L, Chang X, Zheng P, Liu Y (2008) Cutting edge: broad expression of the FoxP3 locus in epithelial

cells: a caution against early interpretation of fatal inflammatory diseases following in vivo depletion of FoxP3-expressing cells. J Immunol 180(8):5163–5166

47. Zuo T, Wang L, Morrison C, Chang X, Zhang H, Li W, Liu Y, Wang Y, Liu X, Chan MW, Liu JQ, Love R, Liu CG, Godfrey V, Shen R, Huang TH, Yang T, Park BK, Wang CY, Zheng P (2007) FOXP3 is an X-linked breast cancer suppressor gene and an important repressor of the HER-2/ErbB2 oncogene. Cell 129(7):1275–1286

48. Zuo T, Liu R, Zhang H, Chang X, Liu Y, Wang L, Zheng P (2007) FOXP3 is a novel transcriptional repressor for the breast cancer oncogene SKP2. J Clin Invest 117(12):3765–3773

49. McInnes N, Sadlon TJ, Brown CY, Pederson S, Beyer M, Schultze JL, McColl S, Goodall GJ, Barry SC (2012) FOXP3 and FOXP3-regulated microRNAs suppress SATB1 in breast cancer cells. Oncogene 31(8):1045–1054

50. Wang L, Liu R, Li W, Chen C, Katoh H, Chen GY, McNally B, Lin L, Zhou P, Zuo T, Cooney KA, Liu Y, Zheng P (2009) Somatic single hits inactivate the X-linked tumor suppressor FOXP3 in the prostate. Cancer Cell 16(4):336–346

51. Li W, Katoh H, Wang L, Yu X, Du Z, Yan X, Zheng P, Liu Y (2013) FOXP3 regulates sensitivity of cancer cells to irradiation by transcriptional repression of BRCA1. Cancer Res 73(7):2170–2180

52. Merlo A, Casalini P, Carcangiu ML, Malventano C, Triulzi T, Menard S, Tagliabue E, Balsari A (2009) FOXP3 expression and overall survival in breast cancer. J Clin Oncol 27(11):1746–1752

53. Ladoire S, Arnould L, Mignot G, Coudert B, Rebe C, Chalmin F, Vincent J, Bruchard M, Chauffert B, Martin F, Fumoleau P, Ghiringhelli F (2011) Presence of Foxp3 expression in tumor cells predicts better survival in HER2-overexpressing breast cancer patients treated with neoadjuvant chemotherapy. Breast Cancer Res Treat 125(1):65–72

54. Hinz S, Pagerols-Raluy L, Oberg HH, Ammerpohl O, Grussel S, Sipos B, Grutzmann R, Pilarsky C, Ungefroren H, Saeger HD, Kloppel G, Kabelitz D, Kalthoff H (2007) Foxp3 expression in pancreatic carcinoma cells as a novel mechanism of immune evasion in cancer. Cancer Res 67(17):8344–8350

55. Ebert LM, Tan BS, Browning J, Svobodova S, Russell SE, Kirkpatrick N, Gedye C, Moss D, Ng SP, MacGregor D, Davis ID, Cebon J, Chen W (2008) The regulatory T cell-associated transcription factor FoxP3 is expressed by tumor cells. Cancer Res 68(8):3001–3009

56. Xia M, Zhao M-Q, Wu K, X-Y Lin, Liu Y, Y-J Qin (2013) Investigations on the clinical significance of FOXP3 protein expression in cervical oesophageal cancer and the number of FOXP3+ tumour-infiltrating lymphocytes. J Intl Med Res 41(4):1002–1008

57. Yoshii M, Tanaka H, Ohira M, Muguruma K, Iwauchi T, Lee T, Sakurai K, Kubo N, Yashiro M, Sawada T, Hirakawa K (2012) Expression of Forkhead box P3 in tumour cells causes immunoregulatory function of signet ring cell carcinoma of the stomach. Br J Cancer 106(10):1668–1674

58. Hao Q, Li W, Zhang C, Qin X, Xue X, Li M, Shu Z, Xu T, Xu Y, Wang W, Zhang W, Zhang Y (2013) TNFα induced FOXP3–NFκB interaction dampens the tumor suppressor role of FOXP3 in gastric cancer cells. Biochem Biophys Res Commun 430(1):436–441

59. Lal A, Chan L, DeVries S, Chin K, Scott G, Benz C, Chen Y-Y, Waldman F, Hwang ES (2013) FOXP3-positive regulatory T lymphocytes and epithelial FOXP3 expression in synchronous normal, ductal carcinoma in situ, and invasive cancer of the breast. Breast Cancer Res Treat 139(2):381–390

60. Karanikas V, Speletas M, Zamanakou M, Kalala F, Loules G, Kerenidi T, Barda AK, Gourgoulianis KI, Germenis AE (2008) Foxp3 expression in human cancer cells. J Transl Med 6:19

61. Miranda-Hernandez DF, Franco-Molina MA, Mendoza-Gamboa E, Zapata-Benavides P, Sierra-Rivera CA, Coronado-Cerda EE, Rosas-Taraco AG, Tamez-Guerra RS, Rodriguez-Padilla C (2013) Expression of Foxp3, CD25 and IL-2 in the B16F10 cancer cell line and melanoma is correlated with tumor growth in mice. Oncol Lett 6(5):1195–1200

62. Fontenot JD, Rasmussen JP, Williams LM, Dooley JL, Farr AG, Rudensky AY (2005) Regulatory T cell lineage specification by the forkhead transcription factor foxp3. Immunity 22(3):329–341

63. Kim J, Lahl K, Hori S, Loddenkemper C, Chaudhry A, deRoos P, Rudensky A, Sparwasser T (2009) Cutting edge: depletion of Foxp3+ cells leads to induction of autoimmunity by specific ablation of regulatory T cells in genetically targeted mice. J Immunol 183(12):7631–7634

64. Droeser RA, Obermann EC, Wolf AM, Wallner S, Wolf D, Tzankov A (2013) Negligible nuclear FOXP3 expression in breast cancer epithelial cells compared with FOXP3-Positive T cells. Clin Breast Cancer 13(4):264–270

65. Courtney AE, C Doherty C, Herron B, McCarron MO, Connolly JK, Jefferson JA (2004) Acute polymyositis following renal transplantation. Am J Transpl 4(7):1204–1207

66. Retraction. Foxp3-positive macrophages display immunosuppressive properties and promote tumor growth (2011). J Exp Med 208 (12):2561

67. Put S, Avau A, Humblet-Baron S, Schurgers E, Liston A, Matthys P (2012) Macrophages have no lineage history of Foxp3 expression. Blood 119(5):1316–1318

68. Mayer CT, Kuhl AA, Loddenkemper C, Sparwasser T (2012) Lack of Foxp3+ macrophages in both untreated and B16 melanoma-bearing mice. Blood 119(5):1314–1315

69. Li F, Yang M, Wang L, Williamson I, Tian F, Qin M, Shah PK, Sharifi BG (2012) Autofluorescence contributes to false-positive intracellular Foxp3 staining in macrophages: a lesson learned from flow cytometry. J Immunol Methods 386(1–2):101–107

70. Devaud C, Westwood JA, John LB, Flynn JK, Paquet-Fifield S, Duong CP, Yong CS, Pegram HJ, Stacker SA, Achen MG, Stewart TJ, Snyder LA, Teng MW, Smyth MJ, Darcy PK, Kershaw MH (2014) Tissues can sculpt the tumor microenvironment to affect responses to therapy. Mol Ther 22(1):18–27

71. Hermans IF, Chong TW, Palmowski MJ, Harris AL, Cerundolo V (2003) Synergistic effect of metronomic dosing of cyclophosphamide combined with specific antitumor immunotherapy in a murine melanoma model. Cancer Res 63(23):8408–8413

72. Dannull J, Su Z, Rizzieri D, Yang BK, Coleman D, Yancey D, Zhang A, Dahm P, Chao N, Gilboa E, Vieweg J (2005) Enhancement of vaccine-mediated antitumor immunity in cancer patients after depletion of regulatory T cells. J Clin Invest 115(12):3623–3633

73. Colombo MP, Piconese S (2007) Regulatory-T-cell inhibition versus depletion: the right choice in cancer immunotherapy. Nat Rev Cancer 7(11):880–887

74. Williams LM, Rudensky AY (2007) Maintenance of the Foxp3-dependent developmental program in mature regulatory T cells requires continued expression of Foxp3. Nat Immunol 8(3):277–284

75. Casares N, Rudilla F, Arribillaga L, Llopiz D, Riezu-Boj JI, Lozano T, Lopez-Sagaseta J, Guembe L, Sarobe P, Prieto J, Borras-Cuesta F, Lasarte JJ (2010) A peptide inhibitor of FOXP3 impairs regulatory T cell activity and improves vaccine efficacy in mice. J Immunol 185(9):5150–5159

76. Morse MA, Hobeika A, Serra D, Aird K, McKinney M, Aldrich A, Clay T, Mourich D, Lyerly HK, Iversen PL, Devi GR (2012) Depleting regulatory T cells with arginine-rich, cell-penetrating, peptide-conjugated morpholino oligomer targeting FOXP3 inhibits regulatory T-cell function. Cancer Gene Ther 19(1):30–37

77. Nair S, Boczkowski D, Fassnacht M, Pisetsky D, Gilboa E (2007) Vaccination against the forkhead family transcription factor Foxp3 enhances tumor immunity. Cancer Res 67(1):371–380

78. Xiao Y, Li B, Zhou Z, Hancock WW, Zhang H, Greene MI (2010) Histone acetyltransferase mediated regulation of FOXP3 acetylation and Treg function. Curr Opin Immunol 22(5): 583–591

79. Liu Y, Wang L, Predina J, Han R, Beier UH, Wang LC, Kapoor V, Bhatti TR, Akimova T, Singhal S, Brindle PK, Cole PA, Albelda SM, Hancock WW (2013) Inhibition of p300 impairs Foxp3(+) T regulatory cell function and promotes antitumor immunity. Nat Med 19(9):1173–1177

80. Nair S, Aldrich AJ, McDonnell E, Cheng Q, Aggarwal A, Patel P, Williams MM, Boczkowski D, Lyerly HK, Morse MA, Devi GR (2013) Immunologic targeting of FOXP3 in inflammatory breast cancer cells. PLoS One 8(1):e53150

81. Bilate AM, Lafaille JJ (2012) Induced CD4+Foxp3+ regulatory T cells in immune tolerance. Annu Rev Immunol 30:733–758

82. Triulzi T, Tagliabue E, Balsari A, Casalini P (2013) FOXP3 expression in tumor cells and implications for cancer progression. J Cell Physiol 228(1):30–35