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Review

The Role of FOXP3 in Human Cancers

ŁUKASZ SZYLBERG[1], DOMINIKA KARBOWNIK[1] and ANDRZEJ MARSZAŁEK[1,2]

[1]*Department of Clinical Pathomorphology, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun, Torun, Poland;*
[2]*Department of Oncologic Pathology, Poznan University of Medical Sciences and Greater Poland Cancer Center, Poznan, Poland*

Abstract. *FOXP3 transcription factor can be observed as the main component of the immune system expressed in regulatory T (Treg) cells that regulate hemostasis and self-tolerance. Moreover, the altered expression of FOXP3 was found in autoimmune diseases, benign tumors and carcinomas. Latest reports indicate that the FOXP3 gene mutation can contribute to carcinogenesis, which can be associated with immune response abnormalities. Infiltration of the Treg cells into tumor cells can be associated with prognosis. Understanding the biology of the FOXP3 gene may be crucial in developing new immunotherapeutics.*

Transcription factor forkhead box P3 (FOXP3) is a member of the forkhead family (1). This factor can be observed as the main component of the immune system expressed in regulatory T (Treg) cells (CD4+/CD25+ or CD4+/CD25–) both in cytoplasm and nucleus (2, 3). Aforementioned cells are the immunosuppressive cells (4) that regulate hemostasis and self-tolerance (5). Treg cells are divided into two types: natural Treg (nTreg) and induced Treg (iTreg) (4, 6). The function and development of the Treg cells is regulated by FOXP3 (lymphocyte FOXP3) (1, 4, 5). According to Müller *et al*. and Grimmig *et al*., FOXP3 is the most specific biomarker of Treg cells (4, 7). but it could be also found in other cells, *e.g*. in B lymphocytes and thymocytes (Table I) (8). Moreover, it was revealed that FOXP3 may be expressed by normal tissues, such as lung, thymus, prostate and breast (2, 9, 10).

Correspondence to: Łukasz Szylberg, MD, Ph.D., Department of Clinical Pathomorphology, A. Jurasz University Hospital No. 1, Sklodowskiej-Curie 9, 85-094 Bydgoszcz, Poland. Tel: +48 525854200, Fax: +48 525854049, e-mail: l.szylberg@cm.umk.pl

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The FOXP3 gene includes 11 coding and 3 non-coding exons. (9) The coding exons encode a 47 kDa protein composed of 431 amino acid (8, 10). FOXP3 protein consists of three α helices (H1, H2, H3) and three β strands (S1, S2, S3). It consists of a N-terminal region, responsible for activation and repression, a central zinc finger and a leucine zipper domain (participate in dimerization) (8), as well as a C-terminal region that includes a forkhead domain (FKH, participation in DNA binding) (Figure 1) (5, 10). According to Chen *et al*. and Tavakoli *et al*., FKH is a critical part of FOXP3 because, as they proved, most of the missense mutations in IPEX syndrome (immune dysregulation, polyendocrynopathy, enteropathy, X- linked) are located in this domain (5, 8).

Human cells synthesize three different isoforms of FOXP3 (Table II) (9, 10). FOXP3FL is a full-length isoform similar to the murine FoxP3. Its molecular weight is 58 kDa. Only this isoform includes exon 2. Exon 2 encodes a domain that inhibits retinoic acid receptor-related orphan receptors (ROR)α and can regulate Th17 differentiation (9, 10). *FOXP3ΔE2* (54kDa) is devoid of exon 2. The consequence of this phenomenon is higher interleukin (IL)-2 secretion and also proliferation because of T-cell stimulation. *FOXP3ΔE2Δ7* is devoid of the exon 2 and the exon 7. The exon 7 encodes a leucine zipper domain. Lack of this domain eliminates the suppression function of Treg cells (9, 10).

Mutation of FOXP3 Gene

In many studies, authors described the altered expression of FOXP3 that was found in cytoplasm and nucleus (1), in autoimmune diseases, benign tumors and carcinomas (Table III). This altered expression was associated with *FOXP3* gene mutations (1, 10, 13).

FOXP3 gene is positioned at the Xp11.23 (2). It is a critical locus because males have only one chromosome X (genotype XY), while females, despite genotype XX, have only one active allele. In consequence, only one hit to the genome could

Table I. *The level of the FOXP3 expression in lymphocytes.*

High level of FOXP3	Low level of FOXP3
CD4+/CD25+ T cells	CD4+/CD25– T cells
CD4+/CD25+/CD8– thymocytes	CD4–/CD8+ T cells
	B cells

Table II. *FOXP3 isoforms.*

Isoform	Structure
FOXP3FL	A full-length isoform
FOXP3ΔE2	Lacks exon 2
FOXP3ΔE2Δ7	Lacks both exon 2 and exon 7

Table III. *Examples of diseases caused by FOXP3 mutations (1, 2, 8-22).*

Cancers	Other diseases
Breast cancer	Allergic rhinitis
Cervix carcinoma	Alopecia areata
Colorectal carcinoma	Crohn's disease
Gastric cancer	Graves' disease
Head and neck carcinoma	Hashimoto's disease
Hepatocellular carcinoma	Inflammatory primary biliary cirrhosis
Lymphoma	IPEX syndrome (immune dysregulation, polyendocrynopathy, enteropathy, X- linked)
Non-small cell lung cancer	Myasthenia gravis
Ovary carcinoma	Psoriasis
Pancreatic carcinoma	Type I diabetes
Prostate cancer	Vitiligo
Small cell lung cancer	
Thyroid carcinoma	
Tongue cancer	

Table IV. *The mechanism of thyroid carcinogenesis.*

High level of FOXP3	Mechanism	Effect
	Inhibition of PPARγ	Proliferation and migration of cells
	Inhibition of caspase-3	Blockade of apoptosis
	Induced expression of NF-κB p65 and cyclin D	

transform a normal cell into tumor cell (10, 13). It can be associated with single nucleotide polymorphism or micro-satellite polymorphism in the *FOXP3* gene (9). The single nucleotide polymorphism is observed in a promoter, introns and coding region. However, microsatellite polymorphism is observed only in the promoter and in the introns' region (9). Two of the most common *FOXP3* genotype polymorphisms are rs3761549 (C>T) and rs3761548 (C>A) located in the promoter. Previous studies proved that changes in *FOXP3* promoter can be associated with carcinogenesis (2), mainly in a group of patients related to tobacco smoking, asbestosis, pollution and viral infection (14, 15).

Changes in the immune system could be associated with carcinogenesis (similarly as in autoimmune diseases) (Table III) (4, 6, 16). The stimulation of T cells by antigen-presenting cells induces FOXP3 expression and the "acquisition" of suppressor function (9). Such mechanism could play a role in the process when cancer cells escape from the antitumor response (11). The expression of FOXP3 in Treg cells is more intensified in the peripheral blood in patients with cancer comparing to control group (10). Moreover, infiltration of Treg cells into tumor cells can be associated with prognosis. In some neoplasms, such as colorectal cancer (7), melanoma (9), non-small and small lung carcinoma (14), high level of the FOXP3 is associated with poor prognosis. However, in breast (9, 10), prostate (9, 17) and gastric cancer (18, 19), the high level of FOXP3 is associated with good prognosis (7, 10). The differences in prognosis of different tumor types are probably related to different function activation of FOXP3 in given cases. *FOXP3* regulates transcription of target genes (up- and down-regulation) (5). If *FOXP3* binds to the promoter and the 5'regulatory region of CTLA4 (encoding CTLA-4 receptor) or IL2RA (encoding CD25 and IL-2 receptor-α) induces up-regulation of these genes. Such interaction causes histone acetylation and transcription of CTLA-4 and CD25 by T cells. On the other hand, if it binds to the promoter of IL-2, IL-7RA or interferon (IFN)γ, this process inhibits acetylation of histones and chromatin remodeling. In consequence, the expression of IL-7R and IL-2 by T cells is blocked (down-regulation) (10).

The FOXP3 Expression in Human Cancers

a) Colorectal Cancer

Patients with colorectal cancer (CRC) have higher levels of T lymphocytes in their blood than healthy control subjects. Moreover, FOXP3 level is higher in colorectal cancer tissues than in normal colorectal tissues (20). Additionally, according to Grimmig *et al.*, the function of the immune reaction depends on the stage of cancer (2, 20). The highest level of $CD4/CD25^+$ is observed in early lesions (UICC I/II) than in advanced lesions (UICC III/IV). High level of FOXP3, IL-10 and transforming growth factor-beta (TGF-β)

Figure 1. *The scheme of the structure of the FOXP3 protein.*

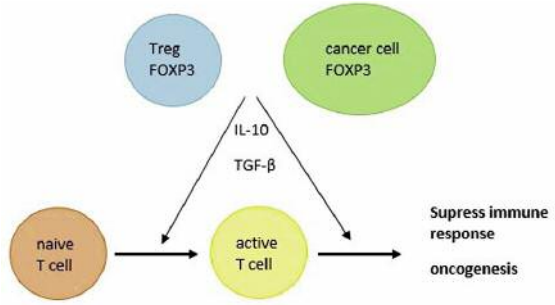

Figure 2. *Cytokines stimulatory effect on oncogenesis. FOXP3 from cancer cells and Treg cells inhibit immune response by interacting with phosphodiesterase 3B, cGMP-inhibited PDE3B and the transcription factor NFκB. For this reason, the inflammation is inhibited by specific cytokines, such as IL-10 and TGF-β (5, 7).*

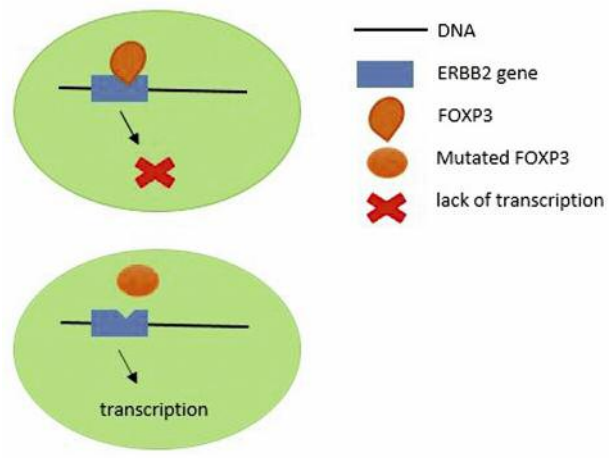

Figure 3. *The effect of the mutation in FOXP3 in breast cancer.*

are characteristic for malignant tumors (7). This finding can indicate that patients with lymph node metastasis have higher level of FOXP3 (UICC III/IV) than patients without metastasis. FOXP3+ Treg cells inhibit immune reaction resulting in immune escape by the tumor (2, 20). Treg cells ($CD4^+$, $CD25^+$) inhibit immune reaction. Treg cells and cancer cells produce cytokines, such as IL-10 and TGF-β (Figure 2). Cytokines inhibit the proliferation of naïve T cells (*in vivo*) and active T cells (*in vitro*) (7). As a result, tumor cell lymph node metastasis is promoted (20).

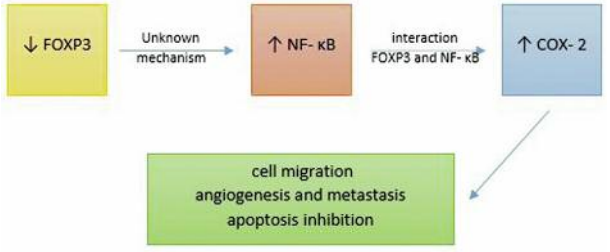

Figure 4. *The mechanism of carcinogenesis in gastric cancer (19).*

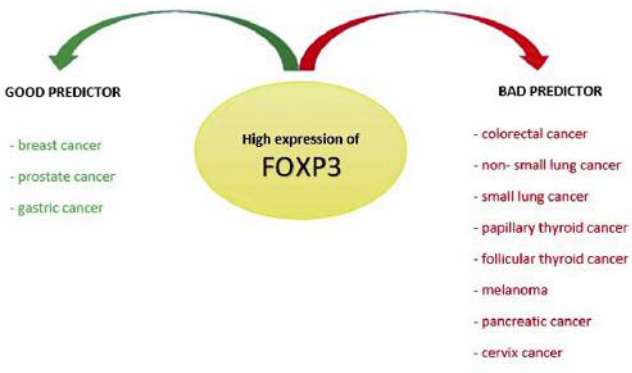

Figure 5. *FOXP3 as a prognostic factor.*

b) Non- small Lung Cancer

Dimitrakopoulos *et al*. analyzed the FOXP3 level in patients with and without non-small lung cancer (NSCLC). They demonstrated low expression of nuclear FOXP3 in normal bronchial epithelium and normal lymphocytes. In contrast, in NSCLC cells and in tumor-infiltrating lymphocytes, FOXP3 is overexpressed (12). Additionally, He *et al*. described dependence between *FOXP3* promoter rs3761548 mutation and NSCLC (14). Moreover, it is possible that, in carcinogenesis, two additional factors participate, prostaglandin E2 (PGE2) and TGF-β. High expression of these factors induces high level of FOXP3 by CD4+/CD25– and $CD4^+/CD25^+$ T- cells. Additionally, PGE2 is necessary to tumor progression, angiogenesis and inhibition of apoptosis (12).

Previous studies show that nuclear FOXP3 level in cancer cells does not correlate with age or sex of the patients, histologic type, stage and tumor grade. However, lymphocyte FOXP3 level is correlated with patients' age (it is higher in patients under 64 years old) (12).

c) Papillary and Follicular Thyroid Cancers

According to Chu *et al*., high level of FOXP3 is associated with thyroid carcinoma. In nodular goiters and follicular adenomas, the level of FOXP3 is low (1, 11, 21). Cunha *et*

al. investigated the association between thyroid cancer and levels of FOXP3. They demonstrated higher level of nuclear FOXP3 in metastatic cancer (1, 21, 22).

Some authors demonstrated that high levels of FOXP3 inhibit expression of PPARγ (nuclear hormone receptor) and caspase-3 (a key pro-apoptotic molecule) and increase expression of nuclear factor-κB (NF-κB) and cyclin D1 (Table IV). The details of this mechanism are unknown. Moreover, high levels of NF-κB and cyclin D induce cell proliferation and migration in thyroid cancer. Additionally, low levels of PPARγ and caspase-3 block apoptosis in cancer cells (11). In conclusion, it could be argued that high levels of FOXP3 decrease PPARγ and indirectly reduce apoptosis of cancer cells.

d) Breast and Prostate Cancer

In mammary tumors, in the process of carcinogenesis, two most important genes participate, *HER-2* and *SKP2*. *HER-2* regulates growth and survival. *SKP2* regulates cell cycle during S and G2 phases by regulated degradation of P27 (9). The high expression of these two genes is related with poor prognosis in patients with mammary cancer (10). On the contrary, high expression of FOXP3 in breast cancer is a good predictor of patients survival (9, 10, 23, 24).

FOXP3 is expressed in normal breast tissues (10). Wild-type *FOXP3*, in normal mammary cells, is bound to and represses *HER-2* and *SKP2* genes. It represses transcription of *HER-2* by binding to the specific locus in the 5' *ERBB2* gene promoter (Figure 3) (9, 23). This indicates that FOXP3 is an important tumor suppressor in breast cancer (9). In contrast, mutated *FOXP3* in cancer cells does not play suppressor function and, as a result, *HER-2* and *SKP2* oncogene expression is missing (10, 23).

The same pathomechanism was found in prostate cancer (10). Studies on prostate cancer revealed chromosomal deletion, somatic mutation and epigenetic silencing of *FOXP3* (9, 17). In those cases, *FOXP3* is unable to repress an oncogene *c-MYC* (10). This causes prostate hyperplasia and prostate cancer. Overexpression of FOXP3 was found in 80% cases of prostate cancer. The correlation between FOXP3 and carcinogenesis was demonstrated by Wang *et al*. They revealed that normal prostate cells proliferated slowly and expressed low levels of *c-MYC*, in contrast to neoplastic cells (25).

e) Melanoma

The high expression of FOXP3 is an unfavorable survival factor in patients with melanoma (9). Low expression of FOXP3 is associated with longer patients' survival (26). $FOXP3^+/CD4^+$ T cells accumulate in the vicinity of tumor cells in higher level than in blood in the same patient. These T cells are similar in phenotype in the blood of healthy patients, patients with melanoma and melanoma tissue. The only difference is the expression of CTLA-4 by T cells in melanoma tissues that results in increased suppression function (27). Tan *et al*., however, revealed that FOXP3 in SK-MEL-28 cell line inhibits proliferation, clonogenicity (*in vitro*) and xenograft growth (*in vivo*). Additionally, FOXP3 stimulates expression of genes involved in pigmentation, namely *MLNA, TYR* and *TYRP*. Moreover, FOXP3 stimulates apoptosis (28). This was also confirmed by Redpath *et al*. According to their result, *FOXP3* represses the expression of c-MYC, which controls cell cycle and apoptosis. *FOXP3* indirectly, by *c-MYC* repression, represses *CDK4* and *CCND2* and activates CDK inhibitors, such as CDKN1A and CDKN2B (9). CDKN1A and CDKN2B are specific tumor suppressors (28). Finally, FOXP3 is related to better survival and higher degree of differentiation (9).

f) Gastric Cancer

In gastric cancer, high level of the FOXP3 is a good prognostic factor (18, 19). Ma *et al*. demonstrated that patients with FOXP3-positive tumors have longer survival than patients with FOXP3-negative tumors. Same phenomenon was observed in prostate and breast cancer as FOXP3 inhibits proliferation and migration of cells (11, 18, 19).

One of the key players in carcinogenesis, in gastric cancer, is cyclooxygenase-2 (COX-2) (Figure 4). COX-2 induces angiogenesis, metastasis and causes poor differentiation. Expression of this protein is controlled by NF-κB levels. The details of this mechanism are unknown. Hao *et al*. demonstrated a relationship between COX-2, NF- κB and FOXP3. Low level of FOXP3 is associated with high level of NF-κB and, hence, high COX-2 levels. Some authors suggest that one of the possibilities of carcinogenic activity of this pathway is modification in the *COX-2* locus by interaction between FOXP3 and NF-κB. Additionally, it is possible that NF-κB is a COX-2 co-repressor. Interaction between FOXP3 and NF-κB might reduce binding the NF-κB to the *COX-2* promoter, thus inducting COX-2 expression (19).

g) Pancreatic Adenocarcinoma (Pancreatic Cancer)

There is no expression of FOXP3 in normal pancreatic duct cells (10, 29). However,, the expression of FOXP3 is an unfavorable survival factor in patients with pancreatic adenocarcinoma (10). This is related to a high level of Treg cells in patients' blood, cancer microenvironment and metastasis in local lymph nodes (29, 30). Treg cells mediate immune escape, thus allowing tumor progression (31). The level of the Treg cells correlates with tumor histologic grade and prognosis. Age, sex and tumor size are irrelevant (30).
The mechanism of carcinogenesis in pancreatic cancer is depended on TGF-β2 (but not TGF-β1), which is expressed by cancer cells (29, 32). TGF-β2 secretion results in

increased expression of Treg cells (conversion of naïve T cells into Treg cells (32)), FOXP3 and finally inhibition of the antitumor response (29). This is confirmed by Wang *et al*. in studies of pancreatic adenocarcinoma where the level of the FOXP3 in Treg cells is higher than in healthy people but the level of Th17 in peripheral blood is lower. This disturbed balance between FOXP3 and Th17 is associated with cancer progression (31).

h) Cervix Cancer

High expression of FOXP3 is a bad predictor of survival for patients with cervical cancer. Cervix cancer is preceded by cervical intraepithelial neoplasia/carcinoma *in situ* (CIN I-III/Cis). Mutations in normal cervix cells is caused by human papilloma virus (HPV) (33-35). The main epithelial cell infection by HPV marker is expression of p16INK4a (33, 36). Only a small percentage of cervix cancer is HPV-negative (35).

Luo *et al*. showed high expression of FOXP3 in cervix cancer tissues and metastatic lymph nodes. Additionally, FOXP3 and p16INK4a expression in healthy people is low or does not occur at all (33). This indicates that FOXP3 level is higher in advanced cancer (33, 36, 37). According to Luo *et al*., high expression of FOXP3 is correlated with FIGO stage, lymph nodes metastasis and tumor size. No correlation was demonstrated between FOXP3 expression and differentiation degree, pathological type and patients' age (33, 37). In contrast, in Chao *et al*.'s study, the level of FOXP3 expression and FIGO stage and lymph node metastasis was no statistically significant (36).

The exact FOXP3 role in cervical epithelium carcinogenesis is unknown. It has been shown that *FOXP3* silencing reduces p16INK4a expression. Probably, *FOXP3* stimulates proliferation, inhibits apoptosis, increases cell invasion and reduces cells in S and G_2 phase of the cell cycle (33).

Final Remarks

There are plenty of evidences that *FOXP3* gene mutation can contribute to carcinogenesis. It can control many other proteins and may play important roles in various biological processes, particularly those of the immunological pathway. FOXP3 is a key factor in pathomechanism in which tumor escapes from immune response. This finding may be useful in developing new drugs as those currently employed, such as denileukin diftitox (ONTAK) (10), LMB-2 (10), cyclophosphamide (10), 5-aza-2'deoxycytidine (Aza) (38), hydroxamates, cyclic tetrapeptides, aliphatic acids and electrophilic ketones (38) are under intensive research. Moreover, the level of FOXP3 may be used as a prognostic factor (Figure 5). In the nearest future, FOXP3 may prove to be a crucial protein that can bring interesting clinical implications.

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