FOXP3 Defines Regulatory T Cells in Human Tumor and Autoimmune Disease

Ilona Kryczek, Rebecca Liu, Guobin Wang, Ke Wu, Xiaogong Shu, Wojciech Szeliga, Linhua Vatan, Emily Finlayson, Emina Huang, Diane Simeone, Bruce Redman, Theodore H. Welling, Alfred Chang, and Weiping Zou

Departments of Surgery, Obstetrics and Gynecology, and Medicine, University of Michigan, Ann Arbor, Michigan, and Department of Surgery, Union Hospital, Wuhan, People’s Republic of China

Abstract

Activated T cells may express FOXP3. It is thought that FOXP3 is not a specific marker to define regulatory T cells (Treg) in humans. Here, we examined the functional phenotype and cytokine profile of the in vivo induced FOXP3+ T cells, primary FOXP3+ and FOXP3- T cells in patients with ulcerative colitis and tumors including colon carcinoma, melanoma, hepatic carcinoma, ovarian carcinoma, pancreatic cancer, and renal cell carcinoma. We observed similar levels of suppressive capacity of primary FOXP3+ T cells in blood, tumors, and colitic tissues. Compared with primary FOXP3+ T cells in the same microenvironment, these primary FOXP3+ T cells expressed minimal levels of effector cytokines, negligible amount of cytotoxic molecule granzyme B, and levels of suppressive molecules interleukin-10 and PD-1. Although the in vitro activated T cells expressed FOXP3, these induced FOXP3+ T cells expressed high levels of multiple effector cytokines and were not functionally suppressive. The data reinforce the fact that FOXP3 remains an accurate marker to define primary Tregs in patients with cancer and autoimmune disease. We suggest that the combination of FOXP3 and cytokine profile is useful for further functionally distinguishing primary Tregs from activated conventional T cells. [Cancer Res 2009;69(9):3995–4000]

Introduction

CD4+CD25+ regulatory T cells (Treg) play a significant role in suppressing tumor-associated antigen-specific immunity and control the pathogenesis of autoimmune diseases (1–4). FOXP3 is a useful marker to phenotypically define Tregs in mice. Mouse FOXP3+ Tregs can be induced in vitro and in vivo with transforming growth factor-β (5–7). However, the in vitro activated human T cells may express FOXP3 (8–10). It has been suggested that FOXP3 may not be a specific marker to define Tregs in humans (8, 10). In current investigation, we compared multiple effector cytokine expression and functional phenotype in the in vitro activated FOXP3+ T cells, primary FOXP3+ versus FOXP3− T cells in patients with ulcerative colitis and patients with colon carcinoma, melanoma, hepatic carcinoma, ovarian carcinoma, pancreatic cancer, and renal cell carcinoma. Our data have shown that primary CD4+FOXP3+ T cells are functionally suppressive and do not express effector cytokines, and the in vitro induced FOXP3+ cells are not functionally suppressive and express effector cytokines. The data indicate that FOXP3 accurately defines primary Tregs in patients with cancers and autoimmune disease and that the combination of FOXP3 and effector cytokine profile may be further helpful to functionally distinguish primary Tregs from in vitro activated FOXP3+ conventional T cells.

Materials and Methods

Human subjects and human tissues. We studied previously untreated ulcerative colitis patients (n = 7), patients with epithelial ovarian carcinomas (n = 17), melanoma (n = 12), colon carcinoma (n = 4), renal cell carcinoma (n = 9), hepatic carcinoma (n = 8), and pancreatic cancer (n = 2). Patients gave written informed consent. The study was approved by local institutional review boards. Cells and tissues were obtained from blood, colitic tissues, and tumors as we described (11). The study was approved by the local institutional review boards.

Flow cytometry analysis (fluorescence-activated cell sorting). T cells were stimulated for 4 h with leukocyte activation cocktail in the presence of GolgiStop (BD Biosciences). These cells were first stained extracellularly with specific antibodies against human CD3, CD4, CD8, CD25, and PD-1 (BD Biosciences), fixed and permeabilized with Perm/Fix solution (E-Biosciences), and finally stained intracellularly with anti-interleukin (IL)-2, anti-IL-8, anti-IL-10, anti-granulocyte-macrophage colony-stimulating factor, anti-tumor necrosis factor-α, anti-IFN-γ, anti-granzyme B, and anti-Ki-67 (BD Biosciences) and anti-FOXP3 (eBiosciences). Samples were acquired on a LSR II (BD Biosciences) and data were analyzed with DIVA software (BD Biosciences).

Immunofluorescence analysis. Immunofluorescence analysis was done as described (11, 12). Tissues were stained with mouse anti-human CD4 (1:40 dilution, RPA-T4; BD Biosciences), mouse anti-human CD8 (1:40 dilution, HIT8a; BD Biosciences), and rat anti-human FOXP3 (1:80 dilution, PCH101; eBiosciences) followed by Alexa Fluor 488-conjugated goat anti-mouse IgG1 and Alexa Fluor 568-conjugated goat anti-mouse IgG2 (all 1:2000 dilution; Molecular Probes/Invitrogen). Fluorescence images were acquired by fluorescence microscope (Leica) and analyzed by ImagePro Plus software.

Immunosuppressive assay. CD4+CD25high T cells were sorted with high-speed sorter (FACS Aria; BD Biosciences) from blood, tumors, and colitic tissues. The expression of FOXP3 was initially defined by fluorescence-activated cell sorting (FACS) in the sorted T cells. The number of Tregs added in the immunosuppressive assay was based on the quantification of CD4+FOXP3+ T cells (13). The responder T cells, CD4+CD25− T cells, antigen-presenting cells, and macrophages were sorted with high-speed sorter from peripheral blood in the same healthy donors to high purity (>99%). The expression of FOXP3 was also examined in the sorted CD4+CD25+ T cells (>0.1% FOXP3+ T cells). The immunosuppressive assay was realized as we described (12, 14–16). Briefly, CD4+FOXP3+ T cells (0.1–105/ml) from blood, tumors, and colitic tissues were added into the coculture system containing the same responder T cells (1.8 × 106/ml) and CD4+CD25− T cells with specific antibodies against human CD3, CD4, CD8, CD25, and PD-1 (BD Biosciences), fixed and permeabilized with Perm/Fix solution (E-Biosciences), and finally stained intracellularly with anti-interleukin (IL)-2, anti-IL-8, anti-IL-10, anti-granulocyte-macrophage colony-stimulating factor, anti-tumor necrosis factor-α, anti-IFN-γ, anti-granzyme B, and anti-Ki-67 (BD Biosciences) and anti-FOXP3 (eBiosciences). Samples were acquired on a LSR II (BD Biosciences) and data were analyzed with DIVA software (BD Biosciences).
monocytes (1 × 10^5/mL) in the presence of 2.5 μg/mL anti-human CD3 and 1 μg/mL anti-human CD28 for 3 days. T-cell proliferation was determined by thymidine incorporation. T-cell cytokines were detected in the supernatants with ELISA kit (R&D Systems).

**Induction of FOXP3 in vitro.** Peripheral blood CD4^+CD25^- T cells (5 × 10^5/mL) were sorted to high purity with <0.1% FOXP3 expression and activated with macrophages (2.5 × 10^5/mL) and 50 ng/mL IL-2 in the presence of 7 μg/mL anti-human CD3 and 4 μg/mL anti-human CD28 for 3 days. The expression of FOXP3 and cytokines was analyzed by FACS.

**Results**

**Primary FOXP3^+ T cells in patients with ulcerative colitis and cancer.** We investigated FOXP3^+ cells in patients with colon cancer and ulcerative colitis. We observed similar levels of CD4^+FOXP3^+ T cells in colon cancer (23 ± 6%) and colitic tissues (25 ± 3%; Fig. 1A; Supplementary Table S1). However, the levels of CD4^+FOXP3^+ T cells were significantly higher in colon cancer and colitic tissues than peripheral blood (Fig. 1A and B). Consistent with flow cytometry analysis, immunofluorescence staining revealed that, in colon cancer and colitic tissues, 98% to 99% FOXP3^+ cells were CD4^+ T cells and <1% CD8^+ T cells expressed FOXP3 in both tumor and colitic tissues (Fig. 1A and B; Supplementary Table S1). The expression of CD25 and FOXP3 was overlapping in both colon cancer and colitic tissues (Fig. 1D, left). Consistent with our

**Statistical analysis.** The Wilcoxon rank-sum test was used to determine pairwise differences and the χ² test used to determine differences between groups. P < 0.05 was considered as significant. Differences in phenotype of T-cell subsets were tested with the paired Student’s t test. All statistical analysis was done on Statistica software (StatSoft).
previous observation in human ovarian cancer (12), >90% CD4+CD25^{high} T cells expressed FOXP3 in tumors and colitic tissues (Fig. 1D, right). Similar results were observed in hepatic carcinoma, melanoma, ovarian carcinoma, renal cell carcinoma, and pancreatic cancer (Supplementary Table S1). Altogether, the data indicate that FOXP3 expression is confined to CD4+ T cells, not to CD8+ T cells, and high expression of CD25 labels majority of the FOXP3+ T cells in the microenvironments of human tumor and autoimmune disease.

Suppressor activities of primary CD4+FOXP3+ T cells in peripheral blood, colon cancer, and colitic tissues. We next examined the suppressive capacity of CD4+FOXP3+ T cells from different compartments in patients with colon cancer and colitis. We initially sorted CD4+CD25^{high} T cells and determined the expression of FOXP3 in these sorted CD4+CD25+ T cells (Fig. 1D, right). The suppressive assay was subsequently realized based on the number of CD4+FOXP3+ T cells by using the responder T cells and antigen-presenting cells from the same healthy donors. We observed similar suppressions on T-cell proliferation (Fig. 2A) and IFN-γ production (Fig. 2B) mediated by FOXP3+ T cells sorted from blood, tumors, and colitic tissues. The suppression was dose-dependent (Fig. 2A). Similar results were observed in the six types of human tumors as described above. The data indicate that these primary FOXP3+ T cells are Tregs and functionally similar regardless of their tissue and pathological sources.

Phenotype and cytokine profile of primary CD4+FOXP3+ T cells in tumor and colitic environment. We examined the functional markers of primary CD4+FOXP3+ T cells in tumor and colitic environment. By gating on CD4+ T cells, we observed that 1% to 3% primary FOXP3+ T cells expressed Ki-67, granzyme B, and PD-1 in colitic and colon cancer tissues (Fig. 3A). By gating on primary FOXP3+ T cells, we showed that 4% to 6% FOXP3+ T cells expressed Ki-67, 2% to 4% FOXP3+ T cells expressed granzyme B, and ~10% FOXP3+ T cells expressed PD-1 in colitic and tumor tissues (except ovarian cancer). In ovarian cancer, 19% FOXP3+ T cells expressed PD-1 (Table 1). The expression levels of granzyme B and PD-1 were significantly higher in primary CD4+FOXP3+ T cells and CD8+ T cells in the same compartments (Fig. 3A; Table 1; Supplementary Table S1). The data suggest that granzyme B and PD-1 may not be functionally crucial for Treg-mediated suppression in human tumors and autoimmune disease.

We next compared the effector cytokine profile of primary FOXP3+ and FOXP3- T cells in human tumors and colitis. By gating on CD4+ T cells, 0.1% to 3% primary FOXP3+ T cells expressed

---

**Table 1. Phenotype and cytokine profile of CD4+Foxp3+ T cells in cancer and ulcerative colitis patients**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Ulcerative colitis</th>
<th>Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td></td>
</tr>
<tr>
<td>IL-2</td>
<td>7</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>8 ± 5</td>
<td>8 ± 2</td>
</tr>
<tr>
<td></td>
<td>5 ± 3</td>
<td>6 ± 1</td>
</tr>
<tr>
<td></td>
<td>3 ± 1</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>IL-10</td>
<td>2 ± 1</td>
<td>3 ± 1</td>
</tr>
<tr>
<td></td>
<td>2 ± 1</td>
<td>0.2 ± 1</td>
</tr>
<tr>
<td></td>
<td>3 ± 1</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>9 ± 5</td>
<td>6 ± 2</td>
</tr>
<tr>
<td></td>
<td>6 ± 2</td>
<td>5 ± 2</td>
</tr>
<tr>
<td></td>
<td>5 ± 1</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>Granzyme B</td>
<td>2 ± 1</td>
<td>4 ± 2</td>
</tr>
<tr>
<td></td>
<td>2 ± 1</td>
<td>6 ± 1</td>
</tr>
<tr>
<td></td>
<td>3 ± 1</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>Ki-67</td>
<td>4 ± 2</td>
<td>6 ± 1</td>
</tr>
<tr>
<td></td>
<td>5 ± 1</td>
<td>5 ± 1</td>
</tr>
<tr>
<td></td>
<td>5 ± 1</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>PD-1</td>
<td>10 ± 7</td>
<td>13 ± 6</td>
</tr>
<tr>
<td></td>
<td>5 ± 1</td>
<td>2 ± 3</td>
</tr>
<tr>
<td></td>
<td>1 ± 2</td>
<td>19 ± 8</td>
</tr>
<tr>
<td></td>
<td>8 ± 3</td>
<td>8 ± 3</td>
</tr>
</tbody>
</table>

**NOTE:** Single-cell suspensions were prepared from fresh cancer and colitic tissues. Cells were stained with relevant antibodies. T-cell subsets were analyzed by FACS. Results are expressed as the percent ± SE of targeted proteins in CD4+FOXP3+ T cells.
were observed in CD4+ FOXP3- T cells and CD8+ T cells in the same conditions. These cytokines (Fig. 3). The ratio between FOXP3+ and FOXP3- T cells was 1 to 1. T-cell proliferation was detected by [3H]thymidine incorporation in the activated CD4+ T cells and analyzed by FACS. Results are expressed as the percent of FOXP3+ T cells in CD4+ and CD8+ T cells. 

By gating on FOXP3+ T cells, <10% CD4+FOXP3+ T cells expressed IL-2 and IFN-γ (Fig. 4B, left), and Ki-67, a proliferating marker (Fig. 4B, right). The data suggest that the induced FOXP3+ T cells are not able to inhibit FOXP3+ T-cell activation in the culture system. In further support of this notion, we showed that 50% primary tumor FOXP3+ T cells (Fig. 2A), but not the same amount of the in vitro induced FOXP3+ T cells, inhibited thymidine incorporation in the effector T cells (Fig. 4C). We next quantified and directly compared the effector cytokine expression in primary tumor FOXP3+ T cells and the in vitro induced FOXP3+ T cells. The induced FOXP3+ T cells expressed higher levels of effector cytokines, including IL-2 and IFN-γ, than primary FOXP3+ T cells (Fig. 4D). More than 80% induced FOXP3+ T cells expressed the effector cytokines (IL-2 and IFN-γ) and 6% primary FOXP3+ T cells expressed these two effector cytokines (Fig. 4D). The data indicate that primary FOXP3+ T cells and the induced FOXP3+ T cells are functionally different.

Discussion

Tregs inhibit tumor-associated antigen-specific immunity and control the pathogenesis of autoimmune diseases (1–4). FOXP3 is a useful marker to define Tregs in mice. Given that the in vitro activated human T cells express FOXP3 (8, 10, 17), it is questioned if FOXP3 is a marker for human Tregs. In this report, we have investigated and compared primary FOXP3+ T cells, FOXP3 T cells, and the in vitro induced FOXP3+ T cells in tumor and autoimmune disease, two typical pathologic scenarios in humans.

Our first observation is that 98% to 99% primary FOXP3+ T cells are CD4+ T cells and <1% CD8+ T cells express FOXP3 in colonic tissues and six types of human cancers. These primary FOXP3+ T cells are functionally suppressive Tregs. It is thought that FOXP3+CD8+ T cells may be CD8 Tregs in humans (17, 18). Although FOXP3+CD8+ T cells can be induced in vitro (this report; refs. 8, 17), our data indicate that this subset is a minor population in vivo in patients with cancer and colitis. We have described previously that plasmacytoid dendritic cells induce IL-10+CD8+ Treg in patients with ovarian cancer (19, 20). However, although substantial amount of primary IL-10+CD8+ Treg is detected in patients with ovarian cancer (this report; ref. 20), these primary IL-10+CD8+ Treg do not express FOXP3 (this report). Primary IL-10+FOXP3 CD8+ T cells may be a functionally relevant CD8+ Treg population in human ovarian cancers (19, 20). The biological significance of the minor FOXP3+CD8+ T-cell population remains to be clarified in human tumors.

We have reported previously that human Tregs sorted from tumors, peripheral blood, and tumor draining lymph nodes exhibit multiple effector cytokines, including IL-2 and IFN-γ, more than primary FOXP3+ T cells (Fig. 4D). The data indicate that primary FOXP3+ T cells and the induced FOXP3+ T cells are functionally different.

Cytokine and functional profiles of the in vitro induced FOXP3+ T cells. To examine the cytokine and functional profiles of the induced FOXP3+ T cells derived from FOXP3+ T cells, we sorted CD4+CD25+ T cells from healthy donors to high purity and checked the expression of FOXP3. If the expression of FOXP3 was <0.1%, these cells were used for furthering experiments. We showed that high levels of FOXP3 expression were induced in the activated CD4+ and CD8+ T cells (Fig. 4A). The in vitro induced FOXP3+ T cells were not a distinct population (Fig. 4A). In the presence of 46% induced FOXP3+ T cells (Fig. 4A, top), FOXP3+ T cells highly expressed multiple effector cytokines, including IL-2 and IFN-γ (Fig. 4B, left), and Ki-67, a proliferating marker (Fig. 4B, right). The data suggest that the induced FOXP3+ T cells are not able to inhibit FOXP3+ T-cell activation in the culture system. In further support of this notion, we showed that 50% primary tumor FOXP3+ T cells (Fig. 2A), but not the same amount of the in vitro induced FOXP3+ T cells, inhibited thymidine incorporation in the effector T cells (Fig. 4C). We next quantified and directly compared the effector cytokine expression in primary tumor FOXP3+ T cells and the in vitro induced FOXP3+ T cells. The induced FOXP3+ T cells expressed higher levels of effector cytokines, including IL-2 and IFN-γ, than primary FOXP3+ T cells (Fig. 4D). More than 80% induced FOXP3+ T cells expressed the effector cytokines (IL-2 and IFN-γ) and 6% primary FOXP3+ T cells expressed these two effector cytokines (Fig. 4D). The data indicate that primary FOXP3+ T cells and the induced FOXP3+ T cells are functionally different.

Notably, although there were 28% IL-10+CD8+ T cells in ovarian cancer, these IL-10+CD8+ T cells did not express FOXP3 (Supplementary Table S1). The data indicate that primary Tregs express limited effector cytokines in tumor and autoimmune environments.

Figure 4. Cytokine and functional profile of the in vitro induced FOXP3+ T cells. A and B, human normal CD4+CD25+ T cells or CD8+ T cells were sorted to high purity (<0.1% FOXP3+ cells). T cells were activated as described in Materials and Methods. Expression of FOXP3 and cytokines was detected by intracellular staining and analyzed by FACS. One of five experiments is shown. A, FOXP3 expression is induced in T cells. FOXP3 expression was detected by intracellular staining in the activated CD4+ and CD8+ T cells and analyzed by FACS. Results are expressed as the percent of FOXP3+ T cells in CD4+ and CD8+ T cells. B, cytokine and Ki-67 expression in the FOXP3+ T cells. Effector cytokine and Ki-67 expression was detected in the activated CD4+ T cells by intracellular staining and analyzed with LSR II by gating on CD4+FOXP3+ T cells. Results are expressed as the percent of cytokine-expressing T cells or Ki-67+expressing T cells in CD4+ T cells. C, primary but not the induced FOXP3+ T cells inhibited T-cell proliferation cells. Immunosuppressive assay was done as described in Materials and Methods. The ratio between FOXP3+ and FOXP3- T cells was 1 to 1, T-cell proliferation was detected by [3H]thymidine incorporation on day 3. Mean ± SD cpm (n = 5). P < 0.01, compared with controls (no Tregs). D, cytokine expression in primary and the induced FOXP3+ T cells. Primary colon tumor–infiltrating T cells (n = 7) and the in vitro activated conventional T cells (n = 5; see A, top) were subject to intracellular staining for FOXP3, IL-2, and IFN-γ. Data were analyzed with LSR II by gating on CD4+FOXP3+ T cells. Results are expressed as the percent of cytokine-expressing T cells in CD4+FOXP3+ T cells.

Cytokines including IL-2, IL-8, IL-10, IFN-γ, tumor necrosis factor-α, and granulocyte-macrophage colony-stimulating factor (Fig. 3B). By gating on FOXP3+ T cells, <10% CD4+FOXP3+ T cells expressed these cytokines (Fig. 3B; Table 1). High levels of effector cytokines were observed in CD4+FOXP3+ T cells and CD8+ T cells in the same microenvironment. Similar cytokine profile was observed in the six human tumor types (Fig. 3B; Table 1; Supplementary Table S1).
similar suppressor capacity in the in vitro functional assay (12). CD4+CD25+ T cells isolated from patients with inflammatory bowel diseases, including ulcerative colitis, are functionally suppressive (21–24). Early studies suggest that Tregs may be numerically reduced and functionally defective in vitro in patients with certain autoimmune diseases (25–27). Interestingly, our data indicate that, regardless of the tissue sources, Tregs manifest similar suppressive activities in vitro in patients with ulcerative colitis and cancer. In addition to different disease settings, this apparent discrepancy may be partially due to technical evolution. The initial in vitro suppressive assay was based on the purified CD4+CD25+ T cells (25–27). It is understandable that there may be sizable amount of CD4+CD25+ effector T cells in patients with autoimmune diseases. Therefore, these autoimmune disease-associated CD4+CD25+ T cells may exhibit a reduced suppressor activity (25–27). Now, the specific antibody against human FOXP3 is available. After sorting CD4+CD25(high) T cells, we are able to examine the expression of FOXP3 in these sorted T cells, and our suppressive assays are subsequently realized based on the number of CD4+FOXP3+ T cells. Further, to minimize the potential variations among responder T cells from different donors, we have used the responder T cells from the same healthy donors with minimal levels of FOXP3 expression. Thus, our modified assay may be more reliable to define the “true” Treg suppressor activity. Of course, it remains arguable whether these primary FOXP3+ T cells are equally suppressive in vivo in the different microenvironments including human tumors and autoimmune diseases.

To further define these Tregs in the microenvironments of human tumor and autoimmune disease, we have examined their functional markers and cytokine profiles. Approximately 5% Tregs express Ki-67, a proliferating marker, indicating active but limited Treg proliferation in the microenvironments of human tumor and autoimmune disease. Tregs may induce apoptosis of effector T cells and antigen-presenting cells by granzyme B pathway (28), and B7-H1/ PD-1 pathway inhibits tumor immunity (29, 30). As most of primary Tregs do not express granzyme B and PD-1, the data suggest that these two pathways may not be crucial for Treg functionality in these patients.

Conventional T cells, but not Tregs, are thought to express effector cytokines. Polynuclear cytokine profile (IL-2, IFN-γ, and tumor necrosis factor-α) has been described in effector T cells in infectious diseases (31). However, multiple effector cytokines have not been examined in combination with intracellular FOXP3 expression per single-cell basis in human colitic and tumor tissues. Furthermore, it remains unknown if the cytokine profile has been altered in Tregs in pathologic environment in humans including tumors and autoimmune diseases. We have compared the effector cytokine profile of primary FOXP3+ and FOXP3- T cells in human tumors and colitis. In contrast to CD4+FOXP3+ T cells and CD8+ T cells, primary CD4+FOXP3+ T cells expressed minimal amount of effector cytokines. This observation is consistent with a recent report on patients with melanoma (32). Although the effector cytokine-expressing FOXP3+ T cells are a minor population among primary CD4+FOXP3+ T cells, it may be important to further examine whether this minor population is functionally relevant in tumor and autoimmune disease. Nonetheless, the data indicate that primary Tregs differed from conventional T cells in the context of effector cytokine profile in tumor and autoimmune environments.

Intracellular FOXP3 has been used as a surrogate marker to define Tregs in humans and mice (33–35). Recent studies suggest that FOXP3 may not be a specific marker to define Tregs in humans. In vitro activated T cells express FOXP3 (8, 10). Our work has shown three differences between primary FOXP3+ T cells and the in vitro induced FOXP3+ T cells: (a) primary FOXP3+ T cells are a distinct CD4+ T-cell population. Less than 1% primary CD8+ T cells express FOXP3 in vivo in patients with tumors and colitis. However, high levels of FOXP3 can be equally induced in CD4+ T cells and CD8+ T cells in vitro. The induced FOXP3+ T cells are not a distinct population. (b) Less than 10% primary FOXP3+ T cells express polynuclear cytokines, whereas >80% induced FOXP3+ T cells express polynuclear cytokines. (c) Primary FOXP3+ T cells are functionally suppressive, whereas the induced FOXP3+ T cells are not. Therefore, although FOXP3 alone can not be used to define human Treg cells in the in vitro settings, FOXP3 remains an useful marker to investigate primary Tregs in human pathologies including tumor and autoimmune disease.

In summary, our current report has examined primary FOXP3+ T cells in patients with colitis and tumors. Our data show that primary FOXP3+ T cells are functionally suppressive and barely express effector cytokines per single-cell basis in the microenvironments of human tumor and autoimmune disease. This feature is distinct from the in vitro activated FOXP3+ T cells. The data reinforce the fact that FOXP3 remains an useful marker to define primary Tregs in patients with cancer and autoimmune disease. We suggest that the combination of FOXP3 and multiple cytokine profile is useful for further functionally distinguishing Tregs from activated conventional T cells.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

Received 10/1/08; revised 2/4/09; accepted 2/26/09; published OnlineFirst 4/21/09.

Grant support: National Cancer Institute grants CA123088 and CA099985 (W. Zou) and National Cancer Institute through the University of Michigan’s Cancer Center support grant 5 P30 CA46924.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

References

7. Fantini MC, Becker C, Monteleone G, Pallone F, Galle

www.aacrjournals.org 3999 CancerRes2009;69:(9).May1,2009

Cytokines and FOXP3+ T Cells

Published OnlineFirst April 21, 2009; DOI: 10.1158/0008-5472.CAN-08-3804

Downloaded from cancerres.aacrjournals.org on April 19, 2017. © 2009 American Association for Cancer Research.


Tran DQ, Ramsey H, Shewach EM. Induction of FOXp3 expression in naive human CD4+FOXp3 T cells by T cell receptor stimulation is transforming growth factor-β dependent but does not confer a regulatory phenotype. Blood 2007;110:2983–90.


Fontenot JD, Gavin MA, Rudensky AY. Foxp3 expression accurately defines the population of intratumoral regulatory T cells that selectively accumulate in metastatic melanoma lesions. Blood 2008;112:4953–60.


Cancer Res 2009; 69: (9). May 1, 2009 4000 www.aacrjournals.org

Downloaded from cancerres.aacrjournals.org on April 19, 2017. © 2009 American Association for Cancer Research.
FOXP3 Defines Regulatory T Cells in Human Tumor and Autoimmune Disease

Ilona Kryczek, Rebecca Liu, Guobin Wang, et al.