FOXP3, a novel glioblastoma oncosuppressor, affects proliferation and migration

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ABSTRACT:

The transcription factor FOXP3 plays an essential role in regulatory T cell development and function. In addition, it has recently been identified as a tumor suppressor in different cancers. Here, we report that FOXP3 is expressed in normal brain but strongly down-regulated in glioblastoma (GB) and in corresponding GB stem-like cells growing in culture as neurospheres (GB-NS), as evaluated by real time-PCR and confirmed by immunohistochemistry on an independent set of GB. FOXP3 expression was higher in low-grade gliomas than in GB. Interestingly, we also found that neurosphere generation, a feature present in 58% of the GB that we examined, correlated with lower expression of FOXP3 and shorter patient survival. FOXP3 silencing in one GB-NS expressing measurable levels of the gene caused a significant increase in proliferation and migration as well as highly aggressive growth in xenografts. Conversely, FOXP3 over-expression impaired GB-NS migration and proliferation in vitro.

We also demonstrated using ChiP that FOXP3 is a transcriptional regulator of p21 and c-MYC supporting the idea that dysregulated expression of these factors is a major mechanism of tumorigenesis driven by the loss of FOXP3 expression in gliomas. These findings support the assertion that FOXP3 exhibits tumor suppressor activity in glioblastomas.

INTRODUCTION

The forkhead transcription factor FOXP3 plays an essential role in the development and function of regulatory T cells (Treg), defined as $FOXP3^{+}CD4^{+}CD25^{+}$ T cells [1]. In humans, FOXP3 is present in two isoforms, referred to as a and b, but the functional differences between the two isoforms are still unclear [2, 3]. A recent report found that in melanomas, FOXP3 is also expressed by tumor-reactive $CD8^{+}$ T cells. These lymphocytes do not express regulatory markers and maintain early effector profiles ($CD38^{+}$, T-bet^{+}, $perforin^{+}$) [4]. The expression of FOXP3, however, is not restricted to lymphoid tissues such as the thymus, spleen and lymph nodes. It was recently reported that FOXP3 is expressed in tumor cells from pancreatic carcinoma, breast cancer, melanoma, lung cancer and colon cancer [5]

FOXP3 appears to be a multifaceted factor with seemingly opposite functions in cancer biology. In pancreatic carcinoma and in melanoma, FOXP3 has a tumor-enhancing role through Treg and their effect on tumor tolerance [6, 7]; in ovarian, breast and prostate cancer, FOXP3 has a tumor-suppressing function [8, 9]. In breast and prostate cancer, FOXP3 may modulate

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the Transwell-96 system (BD Bioscience, Qume Drive San Jose, CA, USA) as provided by the manufacturer. After 24 h, migrated cells were stained with crystal violet solubilised with 10% acetic acid.

Immunohistochemistry and immunofluorescence

Paraffin was removed with xylene and the sections were rehydrated in graded alcohol. Antigen retrieval was carried out using preheated target retrieval solution (pH 6.0), and the primary antibodies were incubated overnight. The following antibodies were used: FOXP3 (eBiosciences; 1:40), Ki67 (BD Bioscience; 1:50), and CD3 (1:100; Thermo Scientific, Wyman Street Waltham MA, USA). Single immunostains were performed using a standard immunoperoxidase protocol (Vectastain Elite ABC kit, PK-6100; Vector Laboratories, Inc., Burlingame, CA, USA) followed by a diaminobenzidine chromogen reaction (Peroxidase substrate kit, DAB, SK-4100; Vector Lab). The tumor sections were also stained with hematoxylin and eosin to assess the volume of tumor growth. Bright field combined immunostains were performed using the rat-on-mouse HRP-Polymer Kit (Biocare Medical, Pike Lane Concord, CA, USA) for the detection of FOXP3 or the MACH4 Universal AP Polymer Kit (Bio care Medical) for the detection of CD3 and GFAP. The chromogen reaction was developed by DAB, Ferranti Blue or Alkaline Phosphatase/RED, Rabbit/Mouse (DAKO), and the nuclei were counterstained with methyl green. For the double immunofluorescence analysis, tumor sections were incubated with Alexa Fluo-conjugated antibodies for 1 h, counterstained with DAPI (4′,6-diamidino-2-phenylindole, Sigma), and examined using a LEICA SP2 confocal microscope.

Statistical analysis

Cumulative survival curves were constructed by Kaplan–Meier method (MedCalc 9.3). Statistical comparisons of data sets were performed by Student's two-tailed t-test, and the results were considered significant at $P < 0.05$.

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