Foxp3 EXPRESSION IN HUMAN CANCER CELLS [1]

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Transcription factor forkhead box protein 3 (Foxp3) specifically characterizes the thymically derived naturally occurring regulatory T cells (Tregs). Limited evidence indicates that it is also expressed, albeit to a lesser extent, in tissues other than thymus and spleen, while, very recently, it was shown that Foxp3 is expressed by pancreatic carcinoma. [2]

AIM

This study was scheduled to investigate whether expression of Foxp3 transcripts and mature protein occurs constitutively in various tumor types.

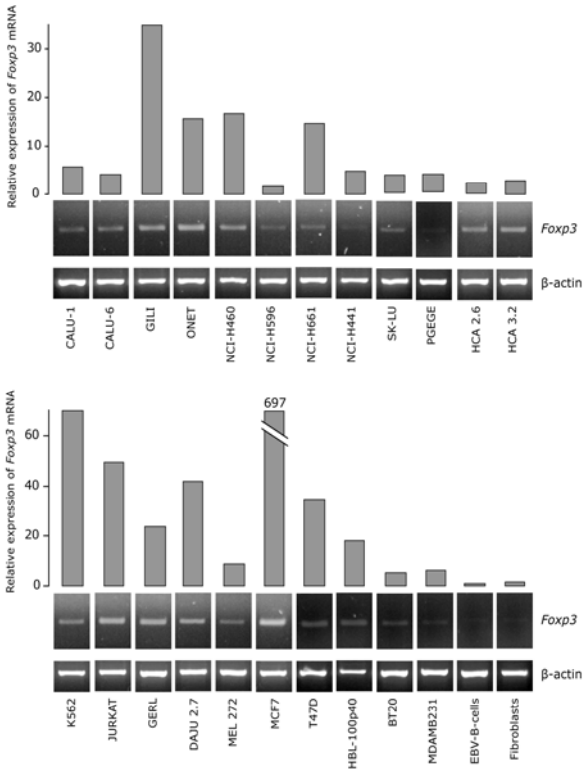
MATERIALS AND METHODS

Twenty five tumor cell lines of different tissue origins (lung cancer: CALU-1, CALU-6, GILI, ONET, SK-LU-1, NCI-H441, NCI-H460, NCI-H596, NCI-H661, NCI-H520, PGEGE, PKAKI, PINTZ; colon cancer: HCA 2.6, HCA 3.2; breast cancer: MCF7, T47D, HBL-100p40, BT20, MDAMB231; melanoma: GERL, DAJU 2.7, MEL272; erythroid leukemia: K562 acute; T-cell leukemia: JURKAT) were studied. Detection of Foxp3 mRNA was performed using both conventional RT-PCR and quantitative real-time PCR while protein expression was assessed by immunocytochemistry and flow cytometry, using different antibody clones.

RESULTS

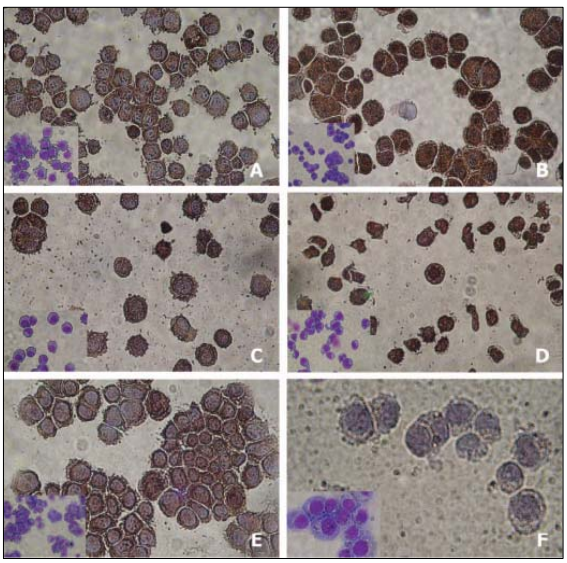
Foxp3 mRNA as well as Foxp3 protein were detected in all tumor cell lines, albeit in variable levels, not related to the tissue of origin. Irrespective of the level of initial expression, culture in the presence of epigenetic drugs did not alter the levels of Foxp3 transcript.

FOXP3 mRNA EXPRESSION



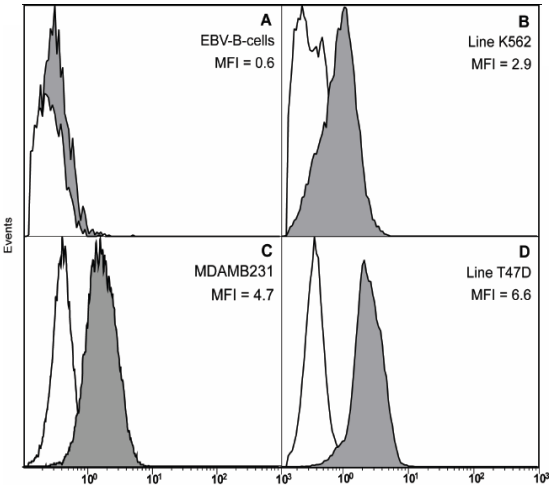
Expression of Foxp3 mRNA in tumor cell lines by qRT-PCR (histograms on the top) and RT-PCR (electrophoretic plots in the bottom).

FOXP3 EXPRESSION DETECTED BY IMMUNOHISTOCHEMISTRY



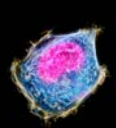
Immunohistochemical staining of tumor cell line cytospins (clone 236A/E7, eBioscience) . A: Melanoma (GERL); predominant cytoplasmic expression. B: Lung adenocarcinoma (GILI); cytoplasmic and nuclear expression. C: Colon adenocarcinoma (HCA 2.6); predominant cytoplasmic expression. D: T Lymphoblastic leukemia (JURKAT); cytoplasmic and nuclear expression. E: Breast adenocarcinoma (MCF7); predominant cytoplasmic expression. F: Not expressing EBV-transformed B cells. Inserts represent May-Gruvald-Giemsa staining of the corresponding cell lines.

FOXP3 PROTEIN EXPRESSION DETECTED BY FLOW CYTOMETRY



Flow cytometric detection of Foxp3 expression in various cell lines (clone PCH101, eBioscience). (A) negative control (EBV-transformed B cells), (B) an erythroid leukemia cell line with low expression, and (C+D) breast cancer cell lines with moderate and high expression. The white underlaid plot represents staining with the isotype.

We offer evidence that Foxp3 expression, not epigenetically regulated, characterizes tumor cells of various tissue origins. The biological significance of these findings warrants further investigation in the context of tumor immune escape, and especially under the light of current anti-cancer efforts interfering with Foxp3 expression.



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

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