Foxp3 EXPRESSION IN HUMAN CANCER CELLS [1]

08-AB-3616-AACR

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BACKGROUND

The transcription factor forkhead box protein 3 (Foxp3) is considered to be a master control gene of the function of thymically derived naturally occurring regulatory T cells (Tregs). Due to the Tregs lineage specification by Foxp3, its tissue expression primarily by lymphoid tissues (thymus, spleen and lymph nodes) is well documented. Scarce information regarding Foxp3 expression by other normal tissues has also been observed, albeit to a far lesser extent. Induction of Foxp3 expression can occur intrinsically in peripheral Foxp3– T cells, while peripheral activated CD4+CD25– and CD8+CD25– T cells can acquire a regulatory function by expressing Foxp3. Since the factors inducing Foxp3 expression in the above T cell populations remain unknown, we hypothesized that a similar induction could take place in other types of cells such as tumor cells. In support of the above, a very recent publication describes the expression of Foxp3 in pancreatic carcinoma cells providing evidence that this could be an important tumor escape mechanism [2].

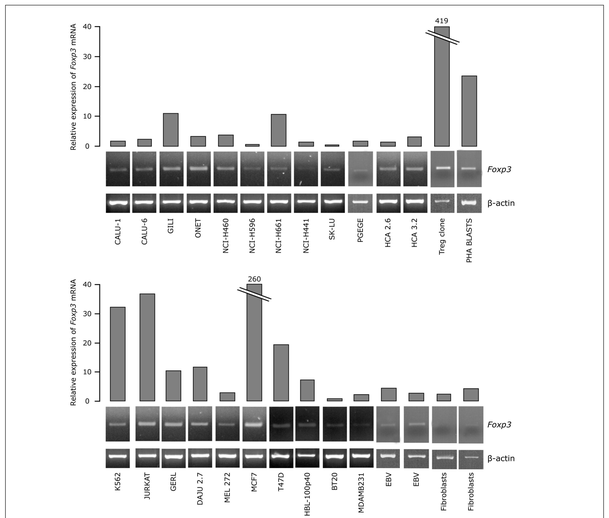
AIM

This study was scheduled to investigate whether expression of Foxp3 transcripts and mature protein is confined to pancreatic carcinoma or can occur constitutively in other tumor types.

MATERIALS AND METHODS

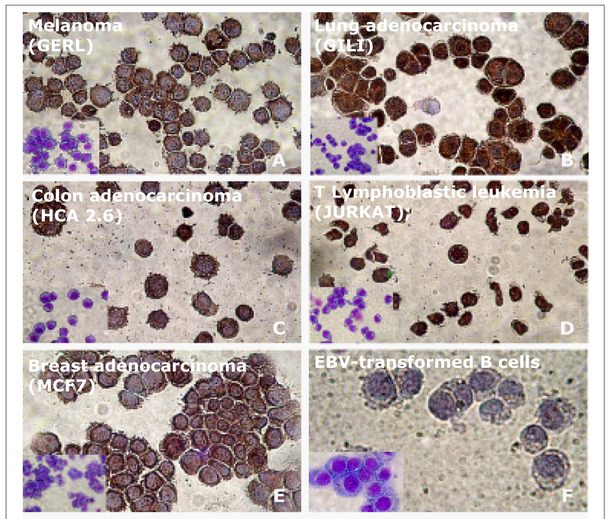
Twenty five tumor cell lines of different tissue origins (lung cancer: CALU-1, CALU-6, GILI, ONET, SK-LU-1, NCI-H441, NCI-H460, NCI-H596, NCI-H661, NCI-H520, PGEGE, PKAKI, PINTZ; colon cancer: HCA 2.6, HCA 3.2; breast cancer: MCF7, T47D, HBL-100p40, BT20, MDAMB231; melanoma: GERL, DAJU 2.7, MEL272; erythroid leukemia: K562 acute; T-cell leukemia: JURKAT) were studied. Detection of Foxp3, IL-10 and TGFb1 mRNA was performed using conventional RT-PCR and quantitative real-time PCR. Foxp3 protein expression was assessed by immunocytochemistry and flow cytometry, using different antibody clones. EBV-transformed B cells were used as negative controls, whereas a CD4+ Treg clone (provided by Dr Lucas, Brussels, Belgium) and PHA blasts were used as positive controls.

RESULTS



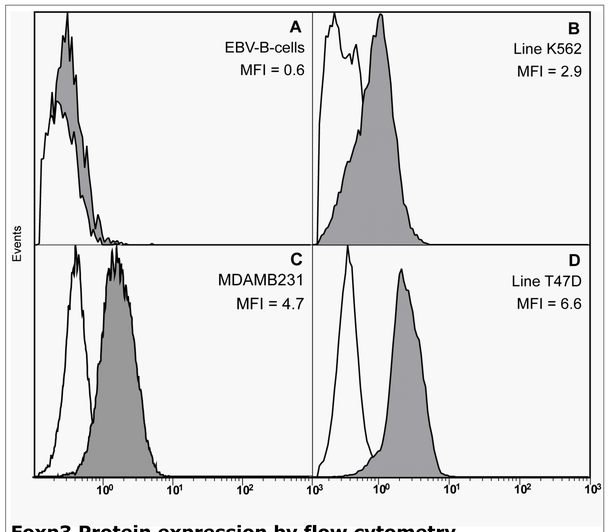
Foxp3 mRNA expression

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



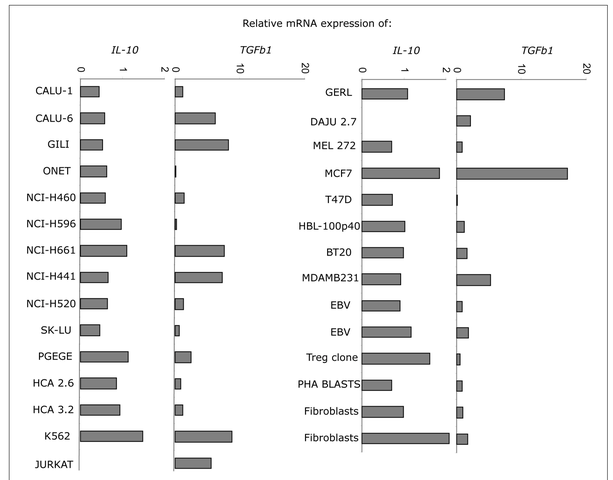
Foxp3 Immunohistochemical staining

Staining of tumor cell line cytospins (clone 236A/E7, eBioscience). Inserts (May-Gruvald-Giemsa staining). A: predominant cytoplasmic expression. B: cytoplasmic & nuclear expression. C: predominant cytoplasmic expression. D: cytoplasmic & nuclear expression. E: predominant cytoplasmic expression. F: Negative control.



Foxp3 Protein expression by flow cytometry

Flow cytometric detection of Foxp3 expression in various cell lines (clone PCH101, eBioscience). (A) negative control, (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 represent staining with the isotype.

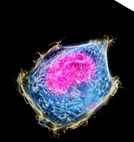


IL10 and TGFb1 mRNA expression

Expression of *IL-10* (left) and *TGFb1* (right) mRNA in tumor cell lines, a Treg clone, PHA blasts, EBV B cells and fibroblasts.

SUMMARY OF KEY FINDINGS

We offer evidence that Foxp3 expression, characterizes tumor cells of various tissue origins. 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. Foxp3 mRNA expression correlated to the expression levels of IL-10 and TGFb1. 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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This work was co-funded by a) a Marie Curie Incoming International Fellowship and a Marie Curie International Reintegration Grant within the 6th European Community Framework Programme (MIF1-CT-2006-021795 IRTALUNG & MIRG-CT-2006-046459 IMMUNOEPIGENETICS), b) a E.U.-European Social Fund (75%) & the Greek Ministry of Development-GSRT (25%)(ENTER04 EP09-8.3.3.) grant, c) a E.U.- European Social Fund (70%) & the Greek Ministry of Development-GSRT (30%) (EPAN05 NON-EU-445) and d) a Glaxo Smith Kline Hellas Research Fellowship (Program No 3116) grant.