Teneurin-4 is frequently expressed in human ovarian carcinomas and is associated with tumor differentiation

[1]Annemarie Ziegler, [1]Rebecca Graumann, [1]Marcos Vásquez, [2]Alejandro Corvalán, [3]Jorge Brañes, [4]Iván Roa, [5]Bernd Wollscheid.
[1]Center for Human Genetics, Faculty of Medicine, Clínica Alemana-Universidad del Desarrollo; [2]Department of Hematology-Oncology and [3]Division of Obstetrics and Gynecology, School of Medicine, Pontificia Universidad Católica de Chile; [4]Division of Pathology, Clínica Alemana de Santiago; [5]Institute of Molecular Systems Biology, NCCR Neuro Center for Proteomics, ETH, Zurich, Switzerland.

Facultad de Medicina
Clínica Alemana - Universidad del Desarrollo
Centro de Genética Humana

OVERVIEW

This work analyzed the expression patterns of teneurin-2 (ten-2) and teneurin-4 (ten-4) in human ovarian tumors and examined potential mechansims involved in control of teneurin gene expression. Teneurin mRNA levels were determined by comparative real-time RT-PCR. The role of DNA methylation on teneurin expression was determined using 5-Aza-Cytidine. We found significantly lower (P<0,001) ten-4 mRNA levels in less differentiated tumors. Teneurin expression could not be induced by demethylating treatment of cell lines. Depletion of ten-2 or ten-4 did not affect proliferation or mRNA abundance of genes related to angiogenesis and apoptosis.

INTRODUCTION

Teneurins are a family of highly conserved transmembrane proteins involved in development of the central nervous system. Using a chemoproteomic approach, we previously identified differential expression of ten-2 in malignant pleural mesothelioma [1], which suggested a potential role of ten-2 as a mesothelioma biomarker. Subsequent experiments demonstrated predominant expression of ten-4 in breast and ovarian cancer cell lines. Although some reports have described aberrant expression of ten-2 and ten-4 in different tumor types, the role of teneurins in human carcinogenesis is currently unknown (reviewed in [2]). Based on our preliminary findings in cell lines, here we analyzed a series of human ovarian carcinomas for expression of ten-2 and ten-4 mRNA. We further examined regulatory mechanisms potentially involved in control of teneurin gene expression.

METHODS

Cell lines were maintained in DMEM supplemented with 10% fetal calf serum, 2 mM glutamine and antibiotics, in a humidified chamber at 37ºC with 5% CO_2. Tumor biopsies and control tissue were obtained from patients undergoing surgery and were stored frozen after stabilization in RNA Later solution (Life Technologies). The study was approved by the participating institutions' Ethical Committees and tissues were obtained with informed consent of patients. Expression of teneurins and other genes was determined by standard RT-PCR and by comparative real-time RT-PCR using a Verity (Applied Biosystems) and an MxPro3005P (Agilent) thermocycler, respectively. Demethylation of cultured cells was achieved by incubation with 5 µM 5-Aza-Cytidine for 72 h.

RESULTS

1. Ten-4 mRNA was detected by RT-PCR in all ovarian tumors and benign lesions, and in a group of normal ovarian samples (***Figure 1***). In comparison, expression of ten-2 was less frequent and mRNA levels appeared to be significantly lower. This was confirmed in real-time PCR experiments which demonstrated reduced expression of ten-2 in ovarian tumors. Ten-4 and the transcription factor EMX2 were co-expressed in all ovarian tissues but not in cell lines derived from other organs.
2. In ovarian and breast cancer cell lines, no correlation could be found between expression of FGF8 or members of the FGFR family, and the presence of ten-2 mRNA (***Figure 2***).
3. Demethylation treatment with 5-Aza-Cytidine for 72 h failed to induce mRNA expression of ten-2 and ten-4 in cell lines (***Figure 3***).
4. In serous ovarian tumors, mean ten-4 but not ten-2 levels were significantly decreased in tumors with poor differentiation (P = <0,001) (***Figure 4***, and not shown).

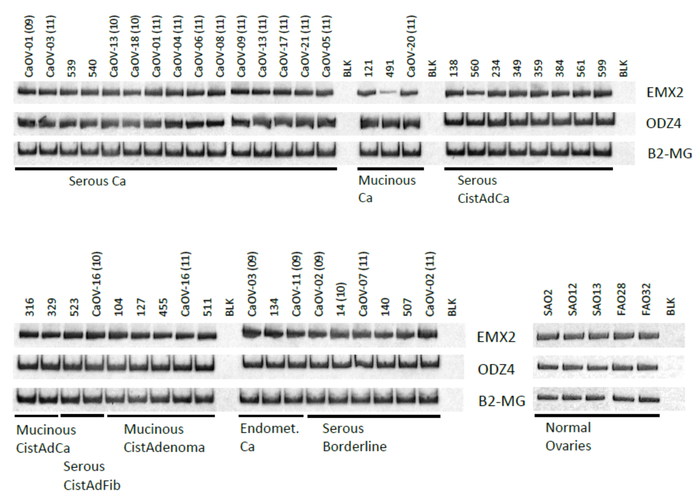

Figure 1. RT-PCR of human ten-4 (ODZ4) and EMX2 in ovarian tissues. β2-microglobulin was used as internal control.

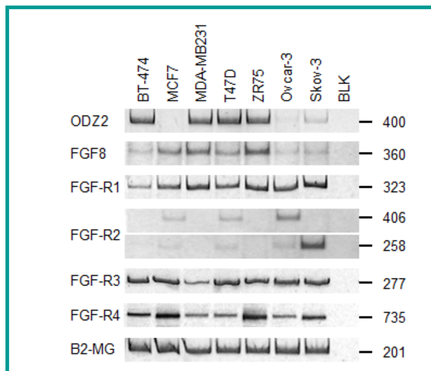

Figure 2. RT-PCR of ten-2 (ODZ2), FGF8 and FGF receptors (FGF-R1 through 4) in human cell lines. β2-microglobulin was used as internal control. Breast cancer (BT474, MCF7, MDA-MB231, T47D and ZR75) and ovarian cancer (Ovcar-3 and Skov3) cell lines were used.

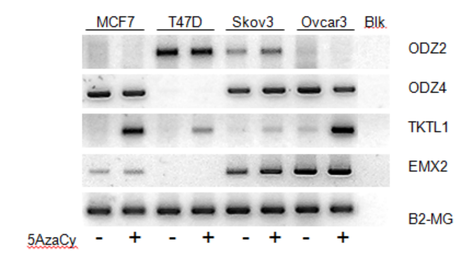

Figure 3. Breast (MCF7, T47D) and ovarian (Skov3, Ovcar-3) cancer cell lines were treated with 5-AzaC for 72 h. Gene expression was measured by RT-PCR. TKTL1 was used as control for demethylation-induced gene expression.

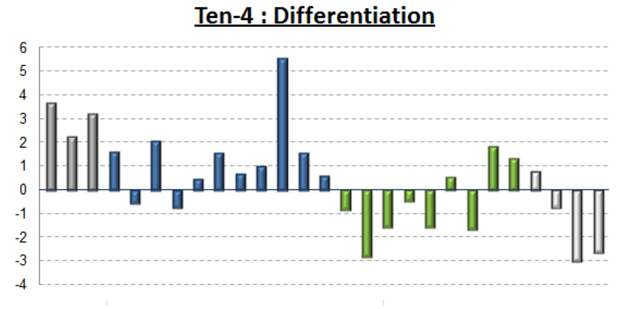

Figure 4. Real-time RT-PCR of ten-4 in human ovarian serous carcinomas. Tumor differentiation was good (grey bars), moderate (blue bars), or poor (green bars). White bars denote undifferentiated tumors. Values are plotted as log2 of fold changes.

CONCLUSIONS

We found ten-4 mRNA expression throughout human ovarian tumors and normal tissues. Ten-4 mRNA showed tissue-specific coexpression with the transcription factor EMX2, suggesting a potential regulatory mechanism as has been reported for other teneurins during embryonic development. In contrast, no evidence was found of regulation of ten-2 by FGF8. Ten-4 mRNA levels were significantly reduced is less differentiated serous ovarian tumors, which could indicate a progressive loss of function with advancing tumor malignity. No comparable trends could be observed for ten-2. In tumors, expression of teneurins appears not to be controlled by epigenetic mechanisms. Experiments into the prognostic utility and functional contribution of ten-2 and ten-4 to the tumor phenotype are currently underway. Some teneurins might harbor potential as tumor biomarkers.

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