9.1 Abstracts and Poster selection

2011


**Neural teneurins are expressed in human tumors and tumor-derived cell lines.**

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Teneurins are a family of transmembrane proteins involved in embryonal development of the central nervous system, where they promote axonal guiding and neural networking. In a previous proteomics-based study, we had identified differential expression of teneurin-2 in malignant pleural mesothelioma versus lung adenocarcinoma, and had proposed that teneurin-2 could be a potential biomarker for tumor discrimination. **Purpose:** We evaluated 12 human tumor-derived cell lines to identify additional tumor types that could express teneurin-2 or teneurin-4. Based on the evidence obtained, we analyzed human ovarian tumors for the presence of teneurin-2 and teneurin-4 mRNA and protein to assess the patterns and frequency of expression. **Methods:** In cell lines, expression of teneurin mRNAs and their transcript variants was assessed by RT-PCR. Teneurin protein expression was detected by immunohistochemistry. Expression of teneurins in frozen tumors and control biopsies was determined by comparative real-time RT-PCR and immunohistochemistry. The study was approved by the participating institutions’ Ethical Committees, and tissues were obtained with informed consent of patients. **Results:** In cell lines, teneurin-2 and teneurin-4 mRNA were expressed in ovarian and breast cancer cells, but infrequently in gastric cancer cells. Characterization of teneurin transcripts in SKOV3 ovarian cells revealed multiple alternative splicing sites, and that expression of exons does not always coincide with the structure postulated in public sequence sources. The presence of teneurin mRNAs correlated with expression of teneurin proteins, as evidenced by immunohistochemical detection. In contrast to cell lines, we found differential expression of teneurins in a preliminary set of 8 ovarian tumors. While expression of teneurin-2 was seldom, teneurin-4 was detected in 6/8 tumors. Immunohistochemistry revealed a membrane localization pattern in tumors but not in surrounding stroma. Experiments are ongoing to analyze additional ovarian tumors and non-malignant controls for presence of teneurin-4. **Conclusions:** We describe for the first time the expression of teneurins in ovarian and breast cancer cell lines. Seventy five percent of human ovarian tumors expressed teneurin-4. Our data suggest that some teneurins might harbor potential as tumor biomarkers. Studies into functional contribution of teneurins to the tumor phenotype are underway. **Supported by Fondecyt 1100605.**


**Neural teneurins are expressed in human tumors and tumor-derived cell lines**

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Introduction: Teneurins are a family of transmembrane proteins involved in development of the central nervous system. We previously detected teneurin-2 expression in malignant mesothelioma and proposed a potential role as tumor biomarker. Purpose: We evaluated 12 human tumor-derived cell lines to identify additional tumor types that express teneurin-2 or teneurin-4. Based on the evidence obtained, we analyzed human ovarian tumors for the presence of teneurin-2 and teneurin-4 to assess the patterns and frequency of expression. Methods: In cell lines, expression of teneurin mRNAs was assessed by RT-PCR. Teneurin protein expression was detected by immunohistochemistry. Expression of teneurins in frozen tumors and control tissues was determined by comparative real-time RT-PCR and immunohistochemistry. Results: In cell lines, teneurin-2 and teneurin-4 mRNAs were expressed in ovarian and breast cancer cells. Multiple teneurin transcript variants could be identified. The presence of teneurin mRNAs correlated with expression of teneurin proteins. In a preliminary set of ovarian tumors, teneurin-4 was detected in 6/8 tumors. Immunohistochemistry revealed a membrane localization pattern in tumors but not in surrounding stroma. Discussion: We describe the expression of teneurins in ovarian and breast cancer cell lines. Seventy five percent of human ovarian tumors expressed teneurin-4. Some teneurins might harbor potential as tumor biomarkers. Supported by Fondecyt 1100605.

Analysis of Teneurin-4 Transcript in Human Cancer Cell Lines

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Abstract. Teneurins are a newly discovered family of transmembrane glycoproteins involved in the development of central nervous system. Recent evidences suggest an aberrant expression of teneurin-4 in some human cancers, such brain tumors, but its functional role in carcinogenesis remains largely unknown. Information about the structure of vertebrate teneurins transcripts is scarce, and detailed structural analysis for human teneurins transcripts have never been reported. We performed investigations to analyze the mRNA structure of teneurin-4 in human cancer cell lines as a first step in the elucidation of teneurin’s role in carcinogenesis. Introduction. The teneurins protein family is highly conserved from invertebrates to vertebrates and man, both regarding domains architecture and amino acid sequences. Moreover, the teneurins expression patterns are conserved across phyla, suggesting that their function is also maintained. Teneurins are putative transmembrane type II glycoproteins that could be substrate of intracellular tyrosine-kinases, however little is known about both, their potential as signalling transducer and their intracellular pathway. Nevertheless, vertebrate teneurins are expressed in developing CNS and in other non-CNS tissues where may regulate the patterning during morphogenesis as well as at site of cell migration and muscle attachment points. ODZs genes encode for the teneurin proteins. In human, there are four ODZs genes named ODZ1- to -ODZ4 each of which localize on different chromosomes, however, human teneurins predicted transcripts structure presents a high sequence homology as well. Recently, a study shown an aberrant expression of teneurin-4 in malignant human brain tumors such astrocytomas,
oligodendriomas and glioblastomas in which the teneurin-4 up-regulation was evidenced at mRNA level (microarrays) as well as protein level (IHC and WB)\(^4\). Notably, a preliminary screening performed in our laboratory showed that both teneurin-4 and -2 transcripts are expressed in frozen biopsies from ovarian and breast cancers as well as in cell lines derived from these types of tumors. In some cases, teneurin-4 was also detected in normal ovarian tissues adjacent to the tumor, which could indicate that teneurin-4 might be a tissues-specific marker\(^5\). **Aims of study.** In the first part of project we are interesting to confirm the ODZ4 expression in breast and ovarian cancer cell lines. Secondly we are searching to obtain the full-length ODZ4 transcript. **Material and Methods.** Total RNA was purified from human breast (MCF7, ZR75 and MDA-237) and ovarian (SKOV3) cancer cell lines and reverse transcribed into cDNA with oligo(dT) primers. The expression of predicted mRNA exons and its continuity into the transcript were analyzed by PCR amplifications. HeLA cell line was used as teneurin-4 negative expression control and the cDNA was obtained as previously described. **Discussions and Conclusions.** The teneurin-4 expression was detected at mRNA level both in breast and ovarian cancers cell lines, however, it appear that teneurin transcript is a target of many forms of splicing, since the “type” of expressed transcript varies between different tumors and the histology of the tumor. Human teneurin transcripts have never been characterized at structural level before, so we believe that to better understand the potential contribution of teneurin-4 in the carcinogenesis, first of all, the characterization and the cloning of the transcript is a priority for then carry out protein functional studies. **Acknowledgements.** This project is supported in part by Fondecyt Grand Nº0110605

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**ANALYSIS OF TENEURIN TRANSCRIPT STRUCTURE IN HUMAN CANCER CELL LINES**

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**Introduction.** Teneurins are a newly discovered family of transmembrane glycoproteins involved in the development of the central nervous system. Recent evidence suggests an aberrant expression of teneurin-2 and teneurin-4 in some human cancers, but their functional role in carcinogenesis remains unknown. Information on the structure of vertebrate teneurin transcripts is scarce, and human teneurin transcripts have not been characterized. We performed investigations to analyze the mRNA structure of teneurin-2 and teneurin-4 in human cancer cell lines, as a first step in the elucidation of teneurin’s role in carcinogenesis.

**Materials and Methods.** Total RNA was purified from human breast and ovarian cancer cells lines and reverse-transcribed into cDNA. Expression of predicted exons and potential splicing variants was analyzed by PCR using exon-specific primers for teneurin-2 and teneurin-4. The obtained PCR-products were verified by cloning and sequencing. **Results.** Expression of teneurin-2 and teneurin-4 mRNA was detected in breast and ovarian cancer cell lines. We identified the first and last expressed exons for both genes. In teneurin-2, the first two predicted exons were never expressed. Various splicing variants were identified for both genes. **Discussion.** Teneurin-2 and teneurin-4 mRNA were expressed in human ovarian and breast cancer cell lines. The predicted gene structure did not always match our experimental findings.
As in other species, numerous splicing variants appear to exist in humans. Our results suggest that some members of the teneurin family might be expressed in human cancers. Acknowledgements. This investigation was supported in part by Fondecyt Nº 1100605.

9.2 Publications