

Microarray expression analysis of Dupuytren's contracture

Sandra Kraljevic Pavelic and Ivana Ratkaj

Department of Biotechnology, University of Rijeka, trg braće Mažuranića 10, 51000 Rijeka, Croatia

INTRODUCTION:

Dupuytren's disease (DD) is a connective tissue disorder characterized as nodular palmar fibromatosis that causes permanent contraction of one or more fingers. It has a well understood pathology but its etiology remains elusive. Surgical treatment remains the only gold standard the disease treatment. However, it does not eliminate processes that lead to the disease symptoms appearance, and therefore, about 10 % of patients have recurrence and require a new surgery. Some of the risk factors connected with DD are trauma, diabetes, alcoholism, epilepsy and liver disease. In addition, men are 10 times more likely to develop the contracture and more than 25% of older population will become affected by DD as well. We employed a high-throughput technology, the DNA microarray, to screen the entire genome for the changes in gene expression in diseased tissue as to characterize DD at a molecular level and find those genes that might be involved in of disease pathogenesis. We analyzed the obtained transcriptomics results in a biological context of protein changes revealed by our previous proteomic profiling of DD tissue samples. Important signaling molecules and signaling pathways arose from our analysis that might be central to the DD pathogenesis.

MATERIAL AND METHODS:

Primary cell cultures were established from surgically removed affected and patient-matched unaffected DD fascia. Total RNA was isolated by RNeasy mini kit (Qiagen) and further used for microarray expression analysis (HG-U133A array, Affymetrix). Data was analyzed by the software MAS5, GeneSpring and GENEMAPP that gave a list of 18 differentially expressed genes among the cells grown from affected and unaffected samples. Selected genes were further analyzed by a TaqMan probe based real-time PCR and the results were processed with geNorm, rest-384-beta and comparative C_T method for relative quantification. For the protein status study, Western blot analyzes of cells grown from affected and unaffected tissue samples were performed.

RESULTS AND DISCUSSION:

A total of 10 out of 18 differentially expressed genes that had the same expression pattern as obtained by microarray analyzes were confirmed by real-time PCR. RT-PCR data showed variability in the expression among patients which is expected in working with clinical samples. Gene was considered to be confirmed if in 10 or more patients his expression was the same as on the array.

Proteins encoded by confirmed genes are directly involved in:

- interaction with actin and smooth muscle contracture
- remodulation of extracellularmatrix
- proliferating and anti-apoptotic signals