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GENE EXPRESSION PROFILING OF CARDINAL LIGAMENT IN HONG KONG CHINESE WOMEN WITH PELVIC ORGAN PROLAPSE

Hypothesis / aims of study

Pelvic organ prolapse (POP) is a common distressing pelvic floor dysfunction affecting women of all ages, particularly the elderly. Preliminary data have shown that there might be a relationship between POP, abnormal cardinal ligament collagen metabolism, and differential gene expression. We aim to study the gene expression profile of the cardinal ligament in 14 Chinese women with POP and 3 age-matched normal controls. In this study, high density cDNA microarray was used to determine an integrated, genome-wide picture of changing gene function in the cardinal ligament. From this study, gene changes crucial to the development and progression of POP could be discovered. Further characterization of these molecular targets also allows them to be exploited in the diagnosis and prognosis, as well as treatment of this distressing condition. In addition, the correlations of molecular profiles from individual tissue samples to pathologic features and clinical outcome data hold the promise to better classification of POP, and subsequently improved diagnostic and prognostic information for patient management.

Study design, materials and methods

This was a case controlled observational study to compare the differential gene expression between Chinese women with POP and those without. We performed microarray analysis on individual cardinal ligament biopsy specimens from 14 patients with stage III or lV POP and 3 control subjects without POP. This study has been approved by the institutional clinical ethics committee.

Total RNA from frozen tissue samples was extracted and purified by RNeasy^{TM} Mini Kit (Qiagen, Valene, CA). For microarray analysis, 2 µg of total RNA was used to synthesize complementary DNA which then hybridized on the GeneChip Human Genome U133 Plus 2.0 Array (Affymetrix, Santa Clara, CA) according to the manufacturer's protocol. The arrays were then scanned with the fluorometric scanner. The DNA-Chip Analyzer (dChip) Version 1.3 software was used for scanning and data analysis.

Results

Of the 45,000 total transcripts compared, 6 genes were more than 3-fold over-expressed significantly (p<0.05) in patients with POP compared with control subjects [Table 1]. Of the 6 genes identified, 3 were more than 5-fold over-expressed. No gene was found to be under-expressed in patients with POP. The 6 up-regulated genes included: (1) Fibronectin type III domain containing 1 (FNDC1) (5.69-fold), (2) Olfactomedin-like 2B (OLFML2B) (4.18-fold), (3) periplakin (PPL) (3.26-fold), (4) Immunoglobulin J polypeptides (IGJ) (5.86-fold), (5) Immunoglobulin kappa light chain variable region (5.10-fold), (6) Immunoglobulin kappa constant (IGKC) (4.53-fold).

Interpretation of results

In this study, genes of structural proteins related to extracellular matrix and intermediate filament were found to be over-expressed in patients' cardinal ligament tissues when compared to controls. FNDC1 is one the fibronectin subdomains. Fibronectin is involved in many cellular processes, including tissue repair, cell migration and adhesion, as well as interaction with extracellular matrix proteins. The Arg-Ly-Sp (RGD) amino acid sequences in FNDC1 interact with integrins and modulate cell adhesion. PPL is one of the cytolinker proteins in the plakin family, which includes other proteins for making contact with intermediate filament. PPL has also shown to bind with keratin 8 and vimentin for the formation of intermediate filament [1]. OLFML2B belongs to the olfactomedin family, which is a group of secreted polymeric glycoproteins of uncharacterized functions in human [2].
Genes related to immunoglobulins in the transport and immune system were also found to be over-expressed in patients' cardinal ligament tissues when compared to controls. IGJ is a

linker protein for immunoglobulin monomers IgM and IgA. Unlike other immunoglobulins, IGJ does not contribute to antibody specificity and it participates only in polymer-specific effector functions, such as binding of IgM and IgA to the transport receptors on epithelial cells. IGKC and immunoglobulin kappa light chain variable region constitutes the light chains of an antibody. These results suggest the pathogenesis of POP might involve the immune process.

Concluding message

These data suggest that the molecular profiling differences in cardinal ligament between patients with POP and controls may be related to differential gene expression of proteins related to proteins in extracellular matrix, intermediate filament as well as immunoglobulins in the transport and immune system. These differential gene expressions may be the risk factors or markers for the pathogenesis of POP.

References

[1] Unique role for the periplakin tail in intermediate filament association: specific binding to keratin 8 and vimentin. Exp. Dermatol. 2002; 11: 428-438

[2] Molecular evolution of olfactomedin. Mol. Bio. Evol. 1998; 15: 718-726

Table I: Genes over-expressed in patients with pelvic organ prolapse with controls

Ratio	Common	Description	Map	Accession
5.69	FNDC1	Fibronectin type III containing 1	6q25	AI345957
4.18	OLFML2B	Olfactomedin-like 2B	1q23.3	AW007573
3.26	PPL	Periplakin	16p13.3	NM_002705
5.86	IGJ	Immunoglobulin J polypeptides, linker protein for immunoglobulin alpha and mu polypeptides	4q21	AV733266
5.10		Isolate M5 immunoglobulin kappa light chain variable region mRNA, partial cds	2q11.2	BG485135
4.53	IGKC	Immunoglobulin kappa constant	2p12	AW575927