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PROTEOMIC ANALYSIS OF VAGINAL TISSUES IN WOMEN WITH PELVIC ORGAN PROLAPSE USING TWO-DIMENSIONAL GEL ELECTROPHORESIS AND MASS SPECTROMETRY

Hypothesis / aims of study

The pathophysiology of pelvic floor dysfunction resulting in pelvic organ prolapse (POP) in women is complex. There is an urgent need to identify molecular targets for prevention and early treatment of this disturbing disease. This study was aimed to identify differentially expressed proteins in vaginal tissues from women with POP compared with asymptomatic women.

Study design, materials and methods

Women undergoing surgery for POP stage III-IV by POP-Q[20] with no urinary incontinence were selected as POP group, while continent women without POP or with POP no greater than stage I served as controls. Patients with stress urinary incontinence were excluded. The periurethral vaginal wall tissues of ten pairs of postmenopausal, age-matched POP and control women were collected during operation. Protein extracts of each sample were separated on two-dimensional electrophoresis. We defined significant differential expression between POP tissue and its matched control as 2-fold overexpression or underexpression. Differentially expressed protein spots were in-gel digested and identified by mass spectrometry.

Results

After separation on 2D electrophoresis, there were 27 distinct protein spots whose expressing levels had a difference greater than 2 folds. Among these 27 proteins, 8 were only expressed in POP group, 4 were only in control group and 3 were differentially expressed more than 6 folds between two groups.

We picked up 6 of those distinct spots. These 6 spots were excised and submitted to peptide mass fingerprinting analysis in order to identify the proteins. These 6 spots were identified as 4 proteins. Among the identified proteins, transgelin and cysteine and glycine-rich protein 1 (CRP1) only expressed in POP vaginal wall, but the control group expressed more than six folds of keratin 13 and keratin 1 compared to the POP group.

Interpretation of results

Transgelin is a 22–25 kDa actin-binding protein localized to the cell membrane and cytoplasm and a novel regulator of MMP-9 expression. Human transgelin is characterized by a single calponin homology domain located at the amino terminus and either one *c*alponin-*l*ike *r*epeats (CLR) located at the carboxy terminus[1]. Increased levels of transgelin expression have been correlated with cell differentiation and senescence. The 92-kDa type IV collagenase (MMP-9) contributes to tissue remodeling both in physiology and pathology. MMP-9 is a multifunctional protein. Its functions include cleavage of extracellular matrix components (including elastin, type III, IV, and V collagen), regulating angiogenesis and a cryptic pro-migratory epitope (for endothelial cells) from type IV collagen. It has been recognized that the process of pregnancy, labor, vaginal birth, and transient increases in intra-abdominal pressure can result in injury of pelvic floor supporting tissue. Thus, the supportive structures including vaginal wall experience remodeling for recovery[12-16, 27, 30]. MMP-9 is essential for tissue remodeling. It can metabolize the impaired connective tissue and promote new and normal tissue formation. However, transgilin represses this effectiveness of MMP-9, plus increased levels of transgelin expression have been correlated with cell differentiation and senescence. Thus, overexpression of transgilin in POP vaginal tissue interfere the repairing or synthesis of normal and new tissue so that the supporting tissue can not recover its function. CRP1 is detected in gizzard, stomach, intestine/colon, arteries, and lung, all of which contain substantial amounts of smooth muscle tissue. CRP1 is also present in fibroblasts, which are smooth muscle-like in terms of their protein expression patterns. The available data suggest a role for CRPs (including CRP1) as essential positive regulators of muscle differentiation [2]. In our study, highly expression of CRP1 in POP vaginal tissue suggests that POP vaginal muscle tissue undergoes remodeling.

Keratins 1 (K1) are the predominant cytoskeletal intermediate filaments of epidermal cells during transition from the proliferative to the terminal differentiation stage. K1 is the early-stage differentiation marker. Its expression is up-regulated in the transition of the basal keratinocyte from the basal layer to the spinous layer. Keratin 13 (K13) is the major acidic keratin, which together with K4, its basic partner, is expressed in the suprabasal layers of non-cornified stratified epithelia. Expression of K13 is mucosal-specific. Differentiation in human vaginal epithelium can be characterized by the expression of the keratins 1 and 13 which represent the epidermal and nonkeratinizing pathway in stratified epithelia, respectively. K1 is not detectable at the end of pregnancy, which reflecting a completely suppressed epidermal differentiation [3]. Thereby, K13 and K1 marked down-expression in POP vaginal wall tissue compared with controls in our study may suggest that the differentiation of vaginal stratified squamous epithelium is suppressed in POP. POP vaginal epithelium is possible in the hyperproliferative response.

Concluding message

Our data have shown the feasibility of using a 2D PAGE and MS approach to generate protein expression profiles and identify potential molecular targets for POP diagnostics and therapeutics. We have identified several overexpressed and underexpressed proteins specific to POP. Transgelin and CRP1 were identified for the first time as novel protein targets in POP. Their potential involvement and biological significance in POP may provide new insights into the molecular mechanisms underlying POP.

References

[1] Cell Motil Cytoskeleton 1994; 28: 243-255.
[2] J Cell Biol 1997; 139: 157-168.
[3]. Gynecol Obstet Invest 1990; 30: 94-96.

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