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PROFILING MICRORNA EXPRESSION IN CARDINAL LIGAMENT IMPLICATES ITS ROLE IN 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. Previous studies have shown that there might be a relationship between POP, abnormal collagen metabolism, and differential gene expression. However, little is known about the relationship between miRNA expression and POP. We aim to study the miRNA expression profile of the cardinal ligament in 12 women with POP and 12 age- and parity-matched normal controls. In this study, highly sensitive miRNA microarray was used to determine an integrated, complete picture of miRNA expression profiles in the cardinal ligament. From this study, miRNA changes crucial to the development and progression of POP could be discovered.

Study design, materials and methods

This was a case controlled observational study to compare the differential miRNA expression between Chinese women with POP and those without. We performed miRNA microarray on individual cardinal ligament biopsy specimens from 12 patients, in which two patients with stage II POP, nine patients with stage III POP as well as one patient with stage IV POP, and 12 age- and parity-matched control subjects without POP. Total RNA from frozen tissue samples was extracted by miRNeasy Mini Kit (Qiagen, Valene, CA). For miRNA microarray analysis, 100 ng of total RNA was labeled and hybridized on the Human miRNA Microarray Version 3 (Agilent, USA), which contains probes for 866 human and 89 human viral microRNAs from the Sanger database v12.0, according to the manufacturer's protocol. The arrays were then scanned with the Agilent DNA Microarray Scanner. Scanned image files were then processed in Agilent Feature Extraction 10.3.1 software for feature extraction and quality assessment. GeneSpring GX 11 software was used for data analysis such as fold change and TargetScan analysis.

Results

Of the 866 human and 89 human viral miRNAs compared, 7 miRNAs (hsa-miR-363, hsa-miR-135a, hsa-miR-923, hsa-miR-1471, hsa-miR-483-5p, hsa-miR-765 and hsa1-miR-H1) were aberrantly suppressed ($p<0.05$) in patients with POP compared with control subjects. On the other hand, one miRNA, hsa-miR-204, was found to be significantly over-expressed ($p<0.05$) in POP patients [Table 1].

Interpretation of results

In this study, miRNA differentially expressed in the cardinal ligament in POP patients were found. Further analysis by TargetScan, a software which predicts the miRNA targets, suggested that the 8 differentially expressed miRNAs were predicted to target genes related to POP pathogeneisis, including estrogen receptor 1 (*ESR1*) and estrogen-related receptor gamma (*ESRRG*). In addition, it may target genes of extracellular matrix proteins (ECM) like fibrillin 2 (*FBN2*) and collagen type V, alpha 3 (*COL5A3*). In particular, the down-regulated has-miR-363 might target collagen genes like *COL1A2, COL5A1* and *COL19A1* and integrin genes like *ITGA6, ITGA5* and *ITGAV*.

Concluding message

These data suggest that there are differential miRNA expressions in cardinal ligament of POP patients when compared with controls. These differentially expressed miRNAs might regulate the expression of genes related to estrogen pathways [1], collagen metabolism and translation termination. However, further studies are needed to investigate the relationship between the differentially expressed miRNAs and their potential target genes, which in turn to understand more in their roles contributing to the pathogenesis of POP.

Table I: miRNAs with differential expression in patients with pelvic organ prolapse when compared with controls

Systematic Name	p-value	Fold Change	Regulation
hsa-miR-363	0.0056	2.06	down
hsa-miR-135a	0.0067	2.33	down
hsa-miR-923	0.0067	2.36	down
hsa-miR-1471	0.0179	2.85	down
hsa-miR-483-5p	0.0209	2.02	down
hsa-miR-765	0.0243	2.33	down
hsa-miR-204	0.0496	2.13	up
hsa1-miR-H1	0.0496	2.46	down

Graph I: miRNAs with differential expression in patients with pelvic organ prolapse when compared with controls

References

1. Wu W, Lin Z, Zhuang Z, Liang X. Expression profile of mammalian microRNAs in endometrioid adenocarcinoma. European Journal of Cancer Prevention. 2009; 18:50–55

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What were the subjects in the study?	**HUMAN**
Was this study approved by an ethics committee?	**Yes**
Specify Name of Ethics Committee	**Joint CUHK- NTEC Clinical Research Ethical Committe**
Was the Declaration of Helsinki followed?	**Yes**
Was informed consent obtained from the patients?	**Yes**