

MIR-199A-5P MAY BE CAUSING A DECREASE OF UROTHELIAL INTEGRITY IN CHRONIC BLADDER PAIN SYNDROME AND UROTHELIAL CELL CULTURE

Hypothesis / aims of study

Urothelial leakage is known to be one of the factors involved in the bladder pain syndrome (PBS). In previous studies we demonstrated a down-regulation of mRNA levels of tight junction (TJ) proteins in bladder biopsies from BPS patients. While performing a microRNA screening, we identified 28 microRNAs significantly up-regulated in BPS patients [1]. Six of the up-regulated microRNAs are predicted to have the TJ proteins as target genes. The aim of this study was to determine their role in epithelial integrity and tightness.

Study design, materials and methods

We have characterised an immortalized human ureteral cell line TEU-2, which can differentiate into a multilayer culture [2]. The differentiated multilayered TEU-2 cultures show an increased epithelial tightness due to the establishment of tight junctions in the uppermost layer of the cells, equivalent to the umbrella cells of the bladder. We then tested 3 miRNAs, miR-328, miR-320a and miR-199a-5p, identified in the patients' screening, in this system to validate their influence on the TJ proteins. TEU-2 cells were transfected with the microRNAs and the effects of their ectopic expression on the TEU-2 transepithelial electrical resistance (TEER) were measured every 12 h during 3 days.

Results

During the period of observation, the untreated TEU-2 cells, and cells transfected with Cy³-scrambled miR control, miR-328, or miR-320a reached TEER values higher than 1000 Ωcm^2 , indicating that their TJ formation was unaffected. In contrast, TEU-2 cells transfected with Pre-microRNA miR-199a-5p never became tight (TEER < 500 Ωcm^2). Mir-199a-5p is predicted to target the expression levels of genes encoding the TJ proteins claudin 1, JAM-1 or occludin. The effects of miR-199a-5p on the mRNA and protein levels of these TJ proteins are currently being investigated.

Interpretation of results

MiR-199a-5p transfected cells do not achieve normal cell tightness in culture. As this miRNA is upregulated in patients with BPS, our findings imply a role in vivo for urothelial leakage in patients with BPS.

Concluding message

MiRNAs appear to play a role in the development of BPS in humans.

References

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Was informed consent obtained from the patients?	Yes