104

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UROTHELIAL BARRIER FUNCTION IN INTERSTITIAL CYSTITIS

Hypothesis / aims of study

Interstitial cystitis (IC) is a chronic, debilitating condition that is characterised by urinary frequency and urgency, together with chronic pelvic or perineal pain. The precise aetiology of IC remains unclear but is likely to be multifactorial. Further understanding of the pathophysiology of IC is essential to the development of future treatment strategies. One of the primary functions of urothelium is to act as a passive barrier, preventing the urine from interacting with the underlying tissues. The aim of our study was to investigate whether patients with IC have a defective urothelial differentiation programme leading to an ineffective or 'leaky' urinary barrier that could be a major contributing factor in the development and progression of IC.

Study design, materials and methods

Five cold-cut biopsies were obtained from each of ten patients undergoing cystoscopic examination for IC and other non-inflammatory conditions of the bladder, for example overactive bladder (OAB). Diagnosis of IC conformed to the NIDDK criteria. Full ethical approval and informed consent was obtained prior to the collection of surgical samples. One biopsy from each patient was fixed in formalin and embedded in paraffin wax for immunohistochemical analysis. The remaining samples were used for the isolation and culture of urothelial cells, using a modified protocol for the culture of normal human urothelial (NHU) cells from resection specimens [1,2]. Urothelial cell cultures from IC and OAB patients were compared to NHU cells for a) morphology and growth characteristics, b) differentiation potential in response to activation by peroxisome proliferator activated receptor gamma (PPARγ) [3], c) capacity to develop a transepithelial resistance (TER) using electrophysiological techniques applied to cells grown for seven days on Snapwell™ semi-permeable membranes.

Results

Morphology and growth: We have demonstrated that IC cells grow well in culture and can be passaged and maintained for comparable periods of time to cultures from normal human urothelium. Although cultured IC cells are morphologically similar to NHU cells, we have found that a proportion of IC urothelial cells appear to be enlarged and multinuclear, which could be indicative of failed division.

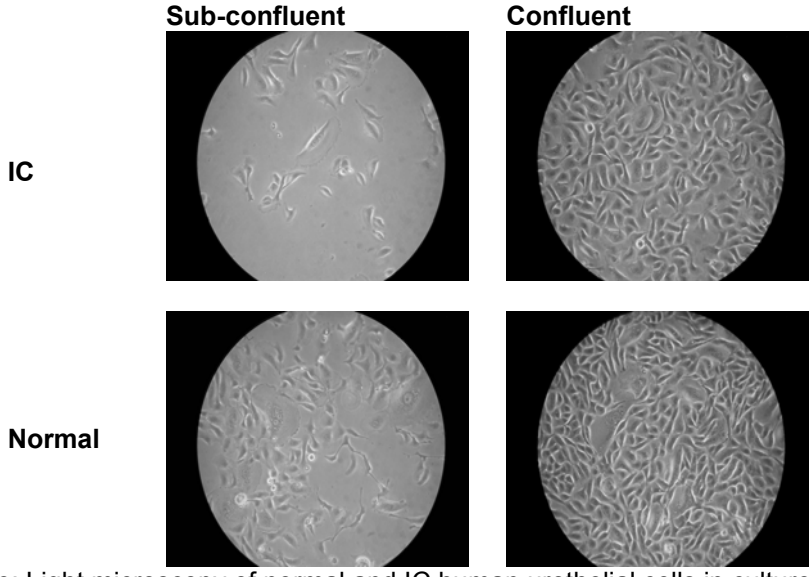

Figure: Light microscopy of normal and IC human urothelial cells in culture.

Differentiation potential:
Initial results indicate that PPARγ-induced differentiation induces the expression of tight-junction proteins, including claudin (CL) 4, CL5 and CL7, in both cultured NHU and IC urothelial cells.

Electrophysiology:
Differentiated NHU cells in culture exhibit a TER of the order of that previously reported for intact mammalian urothelium (rabbit = 3210 ± 800 $\Omega \cdot cm^2$) [4].

	Mean TER ($\Omega \cdot cm^2$)	
	Undifferentiated	**Differentiated**
NHU	53.07 ± 7.38	3614.03 ± 213.09
OAB	65.57 ± 0.39	149.41 ± 49.12
IC	72.21 ± 0.24	117.56 ± 4.8

Interpretation of results
These results demonstrate that IC and OAB urothelial cells can be successfully isolated and cultured from small biopsy samples. Cultured IC urothelial cells display a differentiation potential in response to activation by PPARγ, however, both cultured IC and OAB urothelial cells were unable to form a tight urinary barrier when induced to differentiate, as compared with cultured NHU cells (3614.03 ± 213.09 $\Omega \cdot cm^2$).

Concluding message
These preliminary studies suggest that there are differences between normal and IC urothelium. The functional analyses demonstrate that cultured IC cells do not have the capacity to form an effective urinary barrier. This data suggests that an inherent abnormality of the urothelial cells may be a factor in the aetiopathology of IC.

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