DECREASED NOVEL PHOSPHOKINASE C ISOENZYMES IN MEMBRANES OF BLADDER EPITHELIAL CELL EXPLANTS FROM INTERSTITIAL CYSTITIS PATIENTS

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INTRODUCTION AND OBJECTIVE: Bladder epithelial cells from interstitial cystitis (IC) patients produce significantly decreased levels of heparin-binding epidermal growth factor-like growth factor (HB-EGF) as compared to control cells. However, microarray studies indicated that IC cells do not contain decreased levels of proHB-EGF mRNA, but they do have significantly altered levels of mRNA for at least 13 other proteins, with an overall expression pattern suggesting decreased proliferation and increased differentiation. Certain isoforms of phosphokinase C (PKC) are known to function in the regulation of cell proliferation (α and δ) and/or differentiation (ε), and PKC δ also cleaves proHB-EGF to produce HB-EGF in epithelial cells. We therefore determined whether the quantities of classical (α) and novel (δ and ε) PKC isoforms were altered in the membranes of bladder epithelial cells from IC patients as compared to controls.
METHODS: Epithelial cells were explanted from bladder tissue of 4 patients who fulfilled modified NIDDK criteria for IC and 4 age- and gender-matched asymptomatic controls. Equal concentrations of cytosolic and membrane fractions of cell proteins from each preparation were separated by SDS-PAGE and transferred to nitrocellulose membranes for Western blot analysis using primary antibodies against α, δ and ε isoforms of PKC. Secondary antibodies were labeled with horseradish peroxidase; binding was detected by chemiluminescence and quantified by Storm analysis using Image Quant software.
RESULTS: PKC δ and PKC ε were significantly decreased ($p = 0.017$ and $p = 0.003$) in membrane fractions from IC cells as compared to control cells, while PKC α isoforms were similar in cell membrane fractions from both groups. PKC ε was also significantly increased in the cytosolic fraction from IC cells as compared to controls ($p = .005$), but cytosolic PKC α and δ did not differ significantly between IC and control cells.
CONCLUSIONS: IC bladder epithelial cell explants have significantly decreased quantities of two novel PKC isoforms (δ and ε) in their cell membranes, and significantly increased quantities of PKC ε in their cytosol, as compared to control cells. Because PKC δ functions in cleavage of proHB-EGF to HB-EGF, these findings indicate a potential mechanism by which APF downregulates HB-EGF production. These results also suggest that PKC ε may play a role in the altered gene expression seen in IC epithelial cells.

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