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SELECTION OF SUITABLE INTERNAL STANDARDS FOR MOLECULAR STUDIES IN THE HUMAN BLADDER

Hypothesis / aims of study

The use of molecular techniques (RT-PCR and quantitative competitive RT-PCR) to examine changes in mRNA expression bladder dysfunction is complicated by the choice of internal standard. For an internal standard to be appropriate it must be expressed at a constant level in all tissues being studied and not change with either age or disease. There are a number of housekeeping genes that are commonly used as internal standards: these include the cytoskeletal protein β-actin, the glycolytic enzyme GAPDH and the muscle marker calponin.

The aim of this study was to examine the expression of these housekeeping genes in bladder biopsies from control patients and compare changes in expression with 1) age, 2) gender, 3) anatomical layer (mucosa or detrusor muscle) and 4) region (body wall or trigone).

Study design, materials and methods

Biopsies were collected from 56 female control patients (age range 18 to 86 years) and 54 male patients (age range 30 to 88 years). Patients were undergoing cystoscopy due to a history of carcinoma in situ or asymptomatic haematuria. All control patients displayed normal micturition frequency, with no urgency or detrusor overactivity.

Biopsies were collected into RNALater, dissected into detrusor muscle and mucosa (urothelium + lamina propria) and stored at –70^{o}C until RNA extraction. RNA was extracted using the Epicentre RNA extraction kit followed by two treatments with DNase to remove contaminating DNA.

Expression of β-actin, GAPDH and calponin RNA was determined by RT-PCR. PCR products were separated by agarose gel electrophoresis and quantified by densitometry (pixel density area (mm^2)). All densitometry data were normalised to a standard sample of human RNA (positive control RNA) run on the same gel. Data were expressed as median and interquartile range (IQR) and statistically analysed by one way ANOVA (GraphPad Prism, version 3). Correlations were analysed by linear regression.

Results

Age related changes in expression

There was no age-related change in detrusor β-actin (Fig 1A), nor in detrusor GAPDH (Fig 1C) expression in either males or females. There was also no age-related change in mucosal GAPDH expression (Fig 1D). However, a significant age-related decrease in mucosal β-actin expression was seen in female patients (Fig 1B, P < 0.001). There was also no age-related change in calponin expression in the detrusor (data not shown).

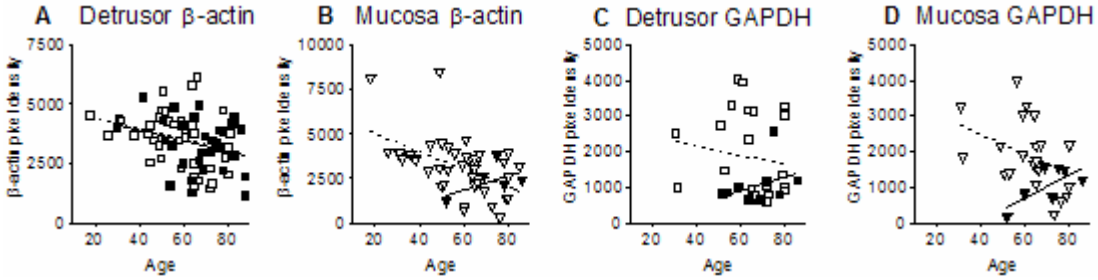

Figure 1. Correlation of expression of housekeeping genes with age in male (solid symbols and lines) and female (open symbols, dashed lines) detrusor (■,□) and mucosa (▼,▽). The r^2 values are: (**A**), β-actin 0.080 (M), 0.089 (F); (**B**), β-actin 0.122 (M), 0.021 (F); (**C**), GAPDH 0.289 (M), 0.263 (F); (**D**), GAPDH 0.495 (M), 0.152 (F).

Gender, anatomical layer and region

In female patients, β-actin expression was not significantly different in body detrusor or mucosa (Fig 2A). Expression in the trigonal mucosa was also similar (pixel density 3057 (1832-3868) n=6). Nevertheless, in male patients, β-actin expression in body mucosa was significantly lower (p<0.001, n=4). However, this has only been examined in a small number of patients and further studies would be needed to verify this finding.

There was no significant difference in the expression of GAPDH in bladder body detrusor and mucosa in either male or female patients (Fig 2B). Although GAPDH expression did appear to change with gender as expression in male patients, in both detrusor and mucosa, was lower than that measured in female detrusor and mucosa (P < 0.01).

The muscle marker calponin was only examined in male patients. As expected, calponin was highly expressed in detrusor muscle (Fig 2C), but not detectable in the body mucosa (n=5).

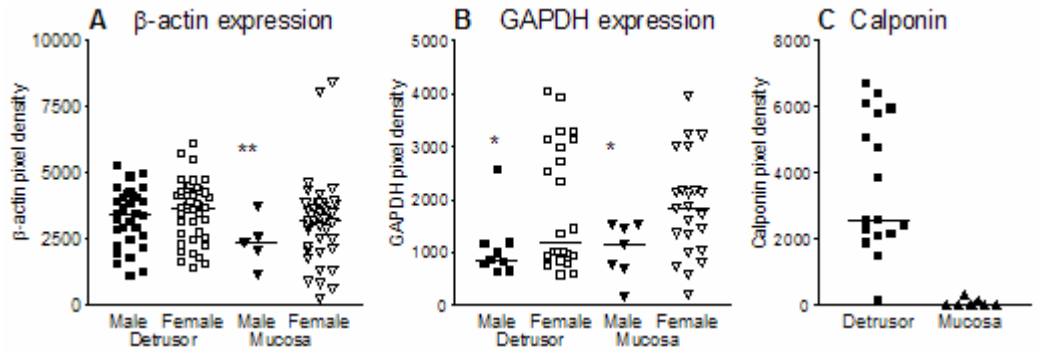

Figure 2. Expression of housekeeping genes in detrusor and mucosa from male and female patients. (**A**) β-actin expression was lower in male mucosa (**P<0.01). (**B**) GAPDH expression was lower in both detrusor and mucosa (* P<0.05) of male patients compared to female detrusor and mucosa respectively. (**C**) In male patients calponin was highly expressed in detrusor and not detected in mucosa. Lines indicate median values.

Interpretation of results

An appropriate internal standard would be a gene that was expressed at a constant level in all tissues being studied, and which is not altered with age, gender and disease. In control patients we have compared expression of two housekeeping genes β-actin and GAPDH. There was a high correlation (P<0.001) of expression between β-actin and GAPDH in human bladder detrusor and mucosa, indicating their usefulness as an internal standard.

Expression of β-actin was similar across different bladder regions. However, it did appear to decrease with age in females (mucosa only). Gender related differences were seen in GAPDH expression in both detrusor and mucosa, although there was no difference in GAPDH expression between regions in the same gender.

Concluding message

The choice of internal control for molecular studies in the human bladder is a complex issue. Large numbers of patients need to be studied and careful consideration should be given to the choice of housekeeping gene used as internal control, since age and gender differences can occur.

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