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ESTABLISHMENT OF IMMORTALIZED HUMAN BLADDER SMOOTH MUSCLE CELLS

Hypothesis / aims of study

Bladder smooth muscle cell (SMC) cultures are an excellent tool for the study of cellular events and molecular mechanisms by which the phenotypic plasticity is controlled. In addition, the establishment of in vitro human bladder SMC systems is required for analysis of pathophysiology and therapeutic approaches of lower urinary tract dysfunction. Although many SMC systems have been previously analysed, human bladder cell biology has not been fully evaluated. Therefore, we attempted to establish the immortalized human bladder smooth muscle cells.

Study design, materials and methods

In immortalization of human smooth muscle cells, primary cultured human bladder smooth muscle cells derived from a 68 years old Caucasian man were transduced with recombinant lentiviruses and retroviruses encoding human cyclin D1, human mutant CDK4, and human telomerase, according to previous report (1). Immunoblotting analysis were performed using primary antibodies included mouse monoclonal antibodies for calponin, high molecular weight caldesmon, α-smooth muscle actin (α-SMA), γ-smooth muscle actin (γ-SMA) and rabbit monoclonal antibodies for muscarinic acetylcholine receptor M2 and M3 and β-cytoplasmic actin (β-CYA) and rabbit polyclonal antibodies for smooth muscle myosin heavy chain 11 (MYH11) and β-tubulin. Secondary antibodies included alkaline phosphatase -labelled antibodies to mouse or rabbit immunoglobulin G. In addition, immunofluorescence analyses for connexin 43 were also performed. Live imaging of intracellular calcium evaluated with Calcium Kit-Fluo 4 after stimulation with high K+ medium and carbachol. Smooth muscle contraction was also evaluated by live imaging of F-actin using SiR-A.

Results

We have established an immortalized human bladder SMC line designated as hBS11. hBS11 cells exhibited continuous cell proliferation for more than 50 populations under optimized culture conditions (Fig. 1). The cell cycle interval was 18.0±3.2h. A chromosome analysis of the cells revealed a normal 46XY diploid karyotype. hBS11 cells expressed smooth muscle differentiation marker proteins, including MYH11, α-SMA, γ-SMA, calponin, h-Caldesmon, β-CYA and β-tubulin. M3 and M2 muscarinic receptor subtypes receptors expressed in differentiated hBS11 cells (Fig. 2). The connexin 43 signals were also detected as spotty signals on the surface of hBS11 cells, and increased during culture (Fig. 3). Both the cholinergic receptor agonist carbachol and a high concentration of extracellular potassium increased intracellular calcium in differentiated hBS11 cells. Differentiated hBS11 cells stimulated by calcium ionophore (A23187) exhibited contractility.

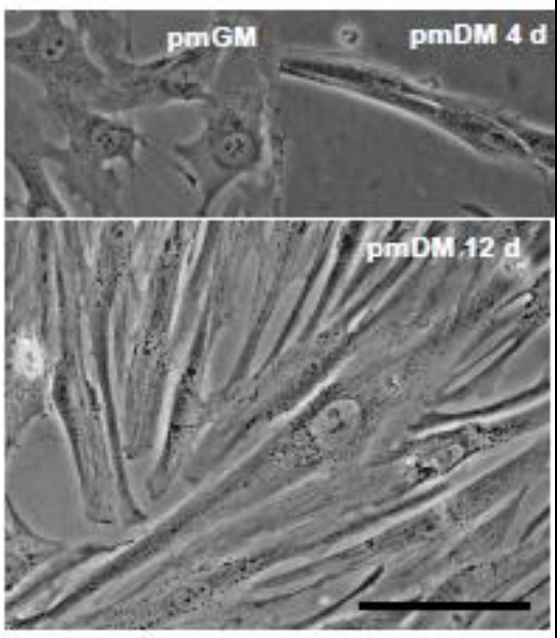
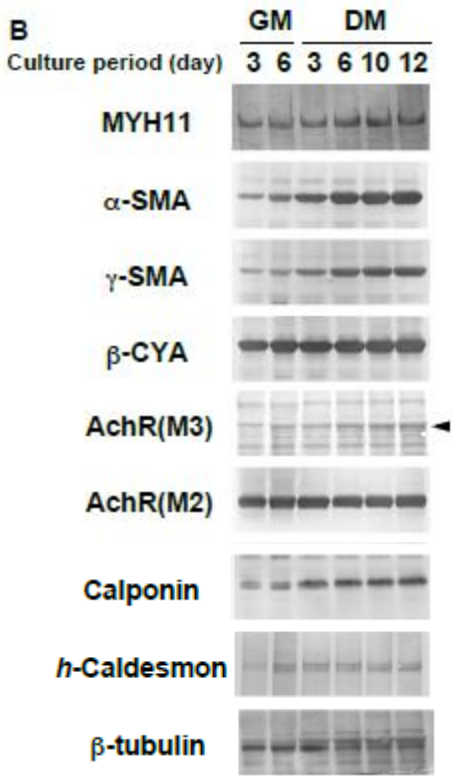


Fig. 1-A: The morphological features of hBS11 cells depended on the culture conditions (4 days and 12 days)



Fig, 1-B: Exponentially growing hBS11 cells expressed smooth muscle differentiation marker proteins, including myosin heavy chain 11 (MYH11), α-smooth muscle actin (α-SMA), γ-smooth muscle actin (γ-SMA), and calponin, h-Caldesmon, β-CYA. M2 and M3 muscarinic receptor subtypes also expressed.

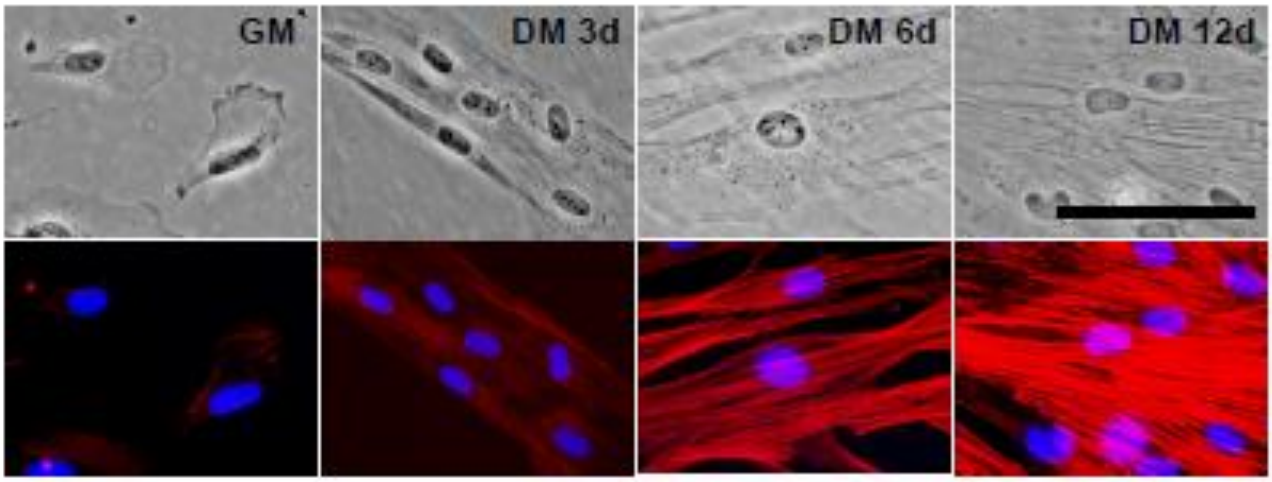


Fig. 2: Actin bundles began to develop in spindle-shaped hBS11 cells within 3 days of culturing, and spread and underwent hypertrophy (6 days and 12 days).

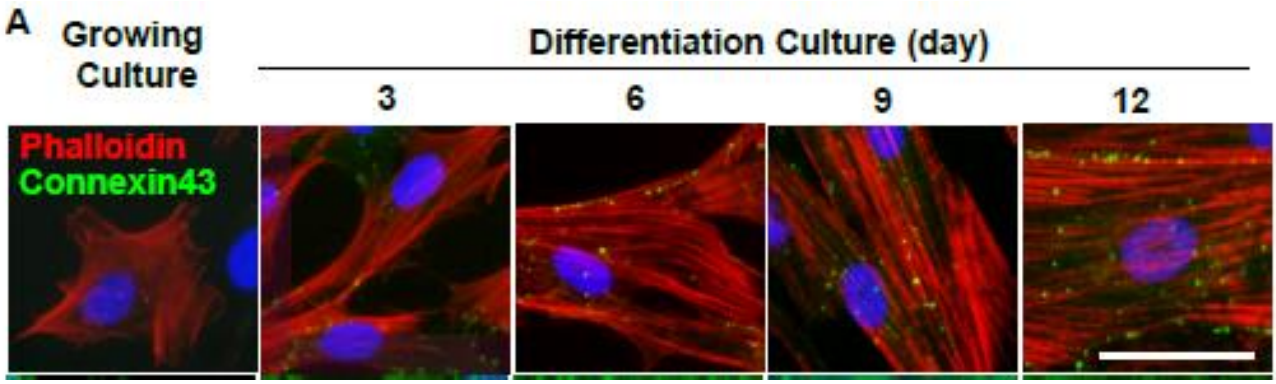


Fig. 3: Specific antibodies against connexin 43 were detected as spotty signals on the surface of hBS11 cells during differentiation

Interpretation of results

The present study demonstrates that the hBS11 cell maintains plasticity of the differentiation that a smooth muscle cell originally has. Furthermore, the present data showed hBS11 cell has functional characteristics of the human bladder smooth muscles cells and potency of the contraction.

Concluding message

In addition to the elucidation of molecular differentiation mechanism, reproduction mechanism, and the function control mechanism of the bladder smooth muscle cells, immortalized hBS11 cell provides useful analytical system for evaluation of the pathophysiological mechanism of lower urinary tract dysfunction and the development of the new treatment options.

References

1. Gene Ther 18(9), 857-66, 2011.

Disclosures

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