

Functional characterization of cells isolated from suburethral mucosa of women with stress urinary incontinence and women with pelvic organ prolapse without incontinence

Flores A¹, Perez-Lorenzo M¹, de la Torre P¹, DeLaPuebla-Ovejero L¹, Alcazar-Garrido A¹, Muñoz-Gállego E^{1,2}, Masero-Casasola A^{1,2}, García-García-Porrero A^{1,2}, Gutiérrez-Vélez M^{1,2}, Medina-Polo J^{1,2}, Grande-García J^{1,2}, García-Muñoz H^{1,2}



1. Instituto de Investigación Hospital 12 de Octubre (imas12), Centro de Investigación, 2. Hospital 12 de Octubre

Introduction

Stress urinary incontinence (SUI) and pelvic organ prolapse (POP) are common worldwide problems that affect the quality of life of millions of women. There are many factors contributing to the development of these disorders such as genetic and ethnicity, age, parity, and other health issues. Situations such as pregnancy, vaginal delivery, overweight and age are the risk factors associated to develop stress urinary incontinence (SUI). Pelvic organ prolapse (POP) was detected in 50% of women with urinary incontinence. There is an obvious need to determine whether there are differences in the etiologies of these two pathologies that are the most common pelvic floor disorders, in order to discover novel therapies, as well as to focus on the preventing factors. Pelvic floor tissues of women with SUI are damaged, at an anatomical, cellular and molecular level.

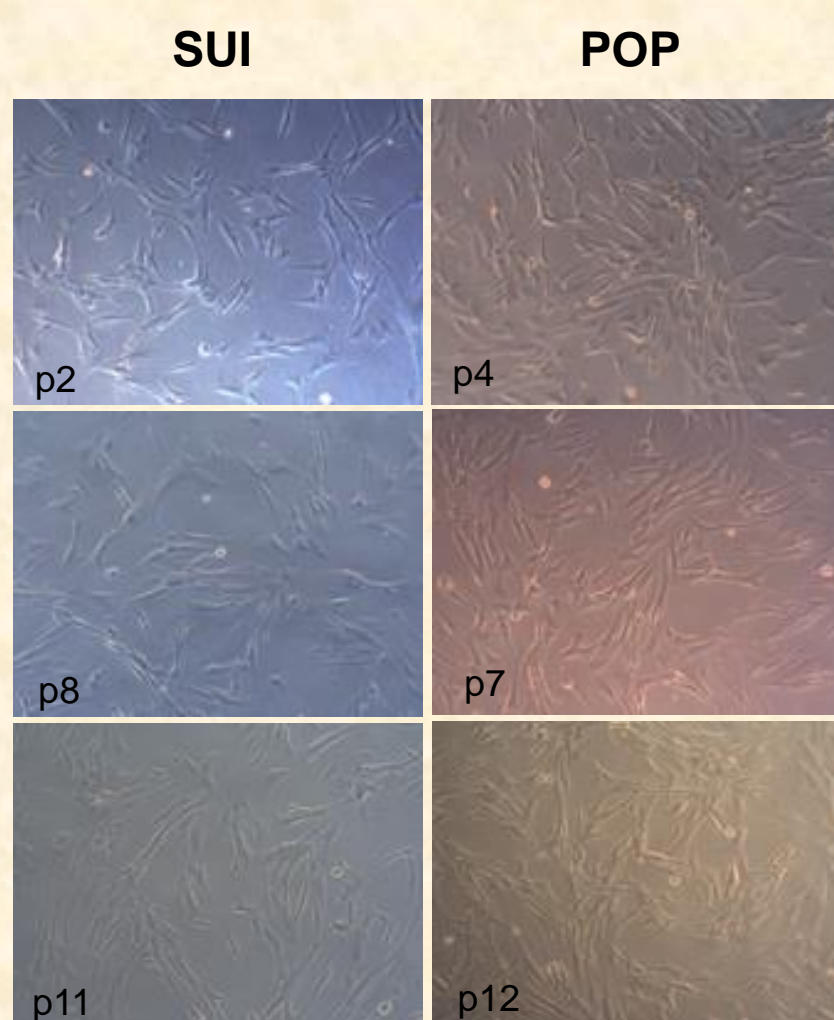
Tissue repair normally occurs very quickly after a trauma, which causes tissue injury. Activated fibroblasts, termed myofibroblasts, respond to damage and play a crucial role in tissue repair. A myofibroblast is a cell with a phenotype between a fibroblast and a smooth muscle cell. Presently, it is accepted that myofibroblasts are fibroblast-like cells that express α -smooth muscle actin (α -SMA), the actin isoform present in typical contractile smooth muscle cells. These myofibroblasts have contractile activity directly related to the presence of α -SMA. These cells are then capable of initiating wound repair by contracting the edges of the wound. Otherwise, desmin is a muscle-specific protein and a key subunit of the intermediate filament in cardiac, skeletal and smooth muscles. Desmin play a critical role in the maintenance of structural and mechanical integrity of the contractile apparatus in muscle tissues.

Our aim was to isolate and characterize fibroblast cells from the suburethral mucosa of patients with stress urinary incontinence (SUI) and pelvic organ prolapse (POP) without incontinence and analyze the existence of differences between them.

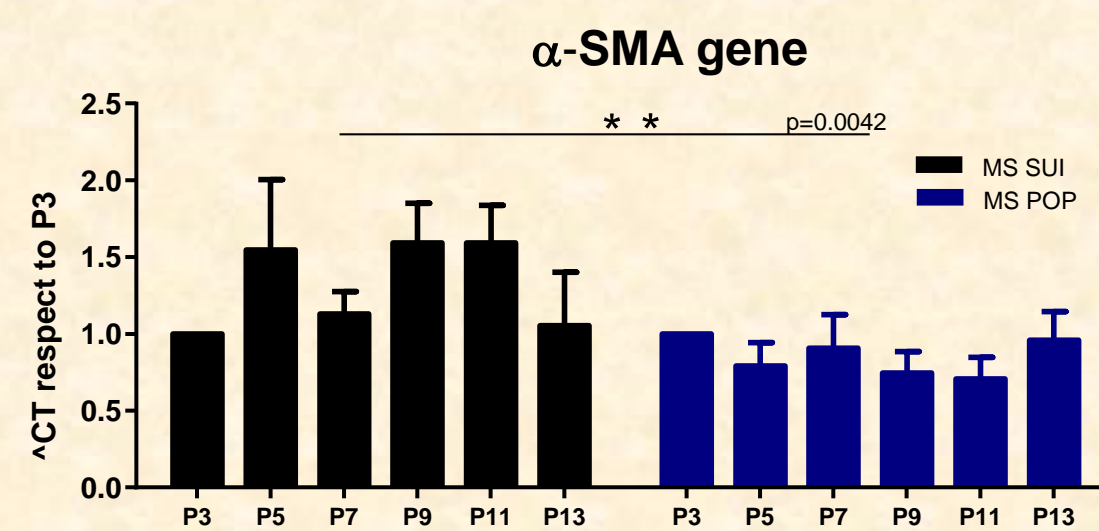
METHODS

Suburethral tissues were obtained from patients with SUI or POP without incontinence under informed consent and cell cultures were established by enzymatic digestion. Isolated cells were cultured in DMEM supplemented with 10% fetal bovine serum. The expression of α -SMA and desmin genes was analyzed by quantitative real-time PCR during the expansion culture. The expression of both proteins was analyzed by immunofluorescence or alphaLISA techniques. Desmin protein was also analyzed in paraffin embedded suburethral tissues by immunocytochemistry. Cell contractibility was measured by CytoSelect™ 24-well Cell Contraction Assay Kit according to the manufacturer instructions

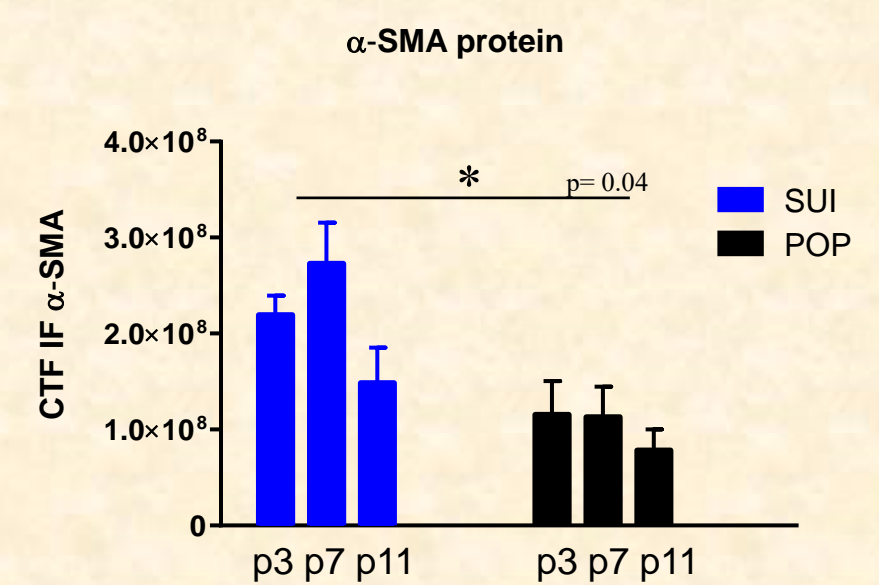
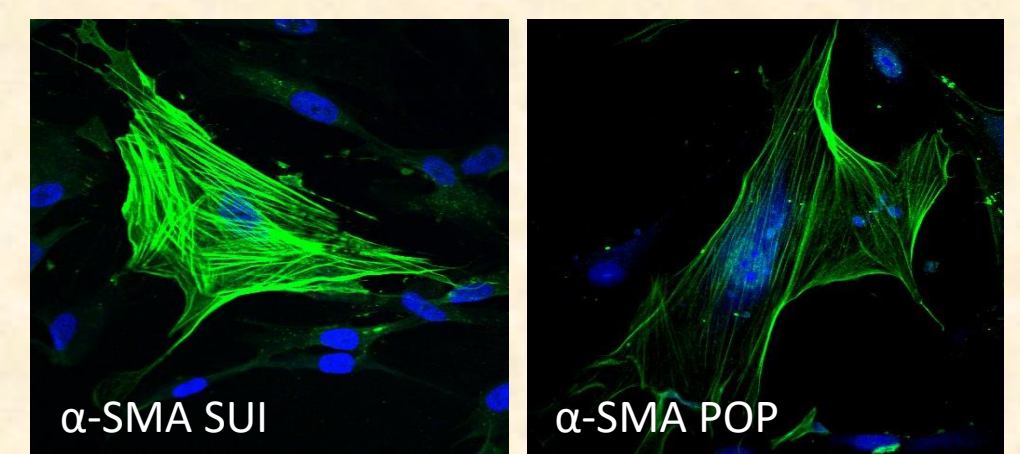
RESULTS



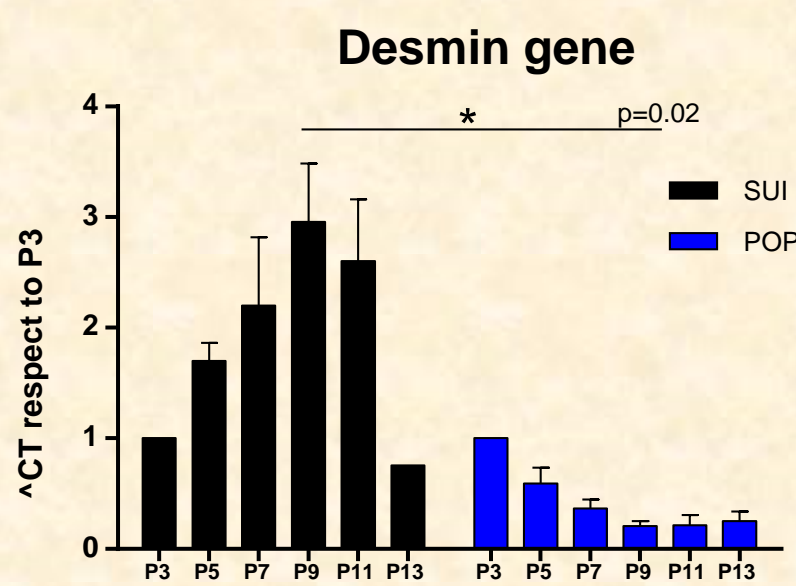
1- The cells obtained from both patients have a fibroblast-like morphology.



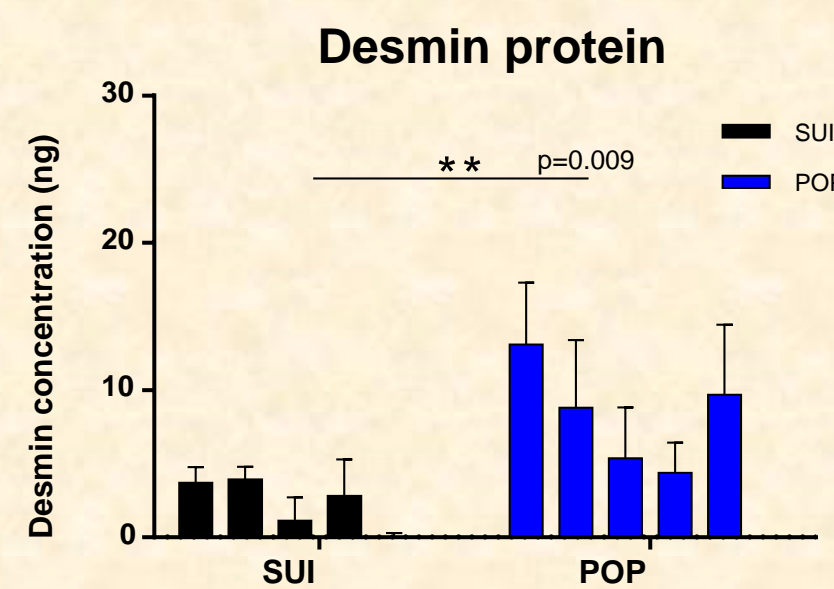
2- The expression of α -SMA gene is greater in SUI cell cultures compared to POP culture. **p=0.0042



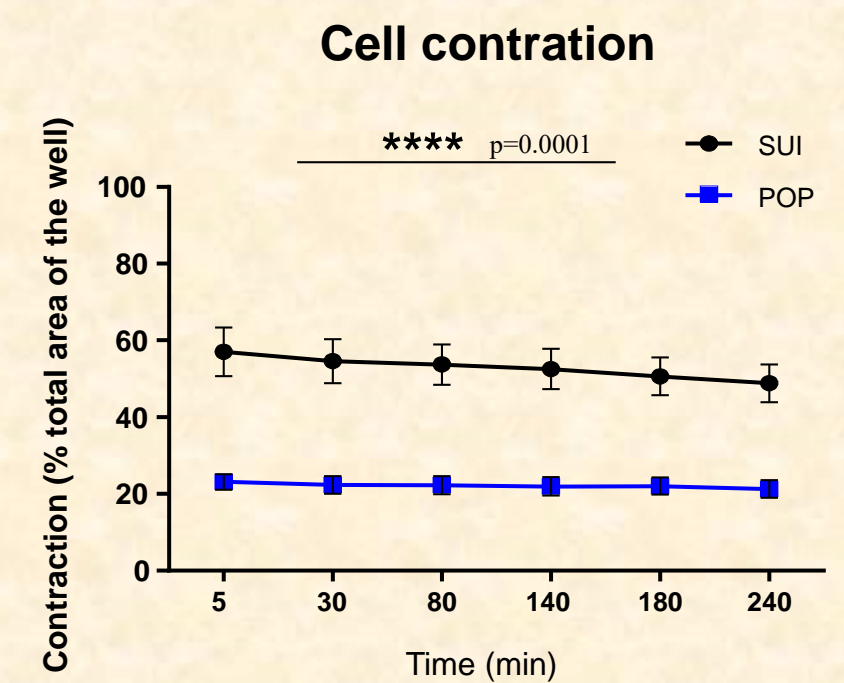
3- Immunofluorescent staining of α -SMA. Higher protein expression of α -SMA was observed in SUI cell cultures as calculated by the corrected total cell fluorescence (CTCF) *p=0.04



4- Basal levels of desmin gene was lower in SUI cells respect to POP cells. However, during the expansion culture the desmin gene is increased in SUI cells until the end of expansion culture while is decreased in POP cells. *p=0.04



5- The amount of Desmin protein was lower in SUI cells respect to POP cells. *p=0.009



6- Cell contraction differences between SUI and POP cells without incontinence. POP cells have higher contraction capability respect to SUI cells. *p=0.0001

CONCLUSIONS

Cells isolated from suburethral mucosa of women with stress urinary incontinence are myofibroblasts with contractile capacity and different to the cells isolated from suburethral mucosa of women pelvic organ prolapse that seem to be a less activated cell type, such as the fibroblast. Our study has shown that there are some phenotypical and functional differences between suburethral cells isolated from patients affected of SUI or POP without incontinence, though to date, we do not know what could be the clinical implication of this. The way in which these cellular differences contribute to the appearance of one pathology and/or the other needs to be elucidated. It is even more relevant taking into account the great percentage of women suffering from both pathologies at the same time.

DISCLOSURES

This work was funded by project PI 15/01803, from the Instituto de Salud Carlos III (Ministry of Economy, Industry and Competitiveness) and cofunded by the European Regional Development Fund Clinical Trial No Subjects Human Ethics Committee Ethics Committee of our Institution (16/275) Helsinki Yes Informed Consent Yes

