

**Disclosures:** Tanvi Potluri (N), Tianming You (N), Ping Yin (N), Jonah J. Stulberg (N), Yang Dai (N), David J. Escobar (N), Hong Zhao (N) and Serdar E. Bulun (N), and Richard L. Lieber (N)

## INTRODUCTION:

Lower abdominal skeletal muscles (LAM) weaken during aging in men due to fibrosis and result in herniation (1). Here, we report utilizing a humanized aromatase mouse model (*Arom<sup>hum</sup>*) that develops spontaneous scrotal hernias, and then investigate the involvement of estrogen receptor alpha (ESR1) in LAM fibrosis and hernia pathology.

## METHODS:

Our prior single-cell RNA sequencing identified *Pdgfra* and *Esr1* as markers of hernia-associated fibroblasts (HAFs) in *Arom<sup>hum</sup>* LAM (2). Here, employing *Pdgfra*-cre mice crossed with *Esr1*-flox, we generated fibroblast-specific ESR1 knockout *Arom<sup>hum</sup>* mice (*fEsr1<sup>-/-</sup>-Arom<sup>hum</sup>*). ESR1 signaling inhibition was achieved via slow-dose Fulvestrant release pellet (0.15mg/kg, 90 days). Hernia size was measured weekly. Isolated HAFs were cultured under estrogen-replete (10nM E2) and deplete (10nM E2 + 100nM Fulvestrant) conditions for multiomics analysis (ERα ChIP-seq, ATAC-seq, and RNA-seq).

## RESULTS:

The *fEsr1<sup>+/-</sup>-Arom<sup>hum</sup>* control mice developed hernias around 5 weeks (Fig 1, **black** squares, n = 5-8 mice/group) while ablation of ESR1 in HAFs entirely prevented herniation (Fig. 1, **green** squares). ESR1 depletion prevented LAM fibrosis and muscle atrophy, emphasizing E2-ESR1 signaling importance for hernia development (data not shown). Fulvestrant treatment led to hernia regression, *reversing fibrosis and muscle atrophy* (Fig. 2, **orange** squares, n = 10-12 mice/group) compared to placebo controls (Fig. 2, **orange** circles). Multiomics analysis of E2 or E2+Fulvestrant-treated HAFs revealed 58 E2/ESR1-modulated genes, including ECM modulators (*Adams6*, *Fbln7*, *Ncam1*, *Ltp1*) and pathways related to cytoskeletal organization, WNT, TGFβ, and Hedgehog signaling. Importantly, LAM tissue from patients having inguinal hernia surgery revealed fibrosis, muscle atrophy, and PDGFRA and ESR1 expression in the stroma (Fig. 3, n = 25 patients). ESR1 and Ki67 expression correlated significantly with fibrosis extent. E2/ESR1 genes from multiomics analysis were also detected in herniated patient's tissue.

## DISCUSSION:

Through the *Arom<sup>hum</sup>* mouse model, we demonstrate that targeted ablation of ESR1 prevents hernia-associated fibrosis, and pharmacological ESR1 inhibition with Fulvestrant reverses hernia and fibrosis, emphasizing the central role that E2/ESR1 plays in fibrosis. The multi-omics analysis of HAFs treated with E2 or E2+Fulvestrant reveals a network of genes and pathways that orchestrate the fibrotic processes, including ECM modulators and cytoskeletal reorganization, corroborating the wide variety of fibrotic mechanisms in skeletal muscle. Validation of ESR1 and PDGFRA expression in human herniated LAM tissues underscores the clinical relevance of our findings, reinforcing the intricate relationship among E2/ESR1 signaling, fibrosis, and herniation. In sum, our study advances our understanding of the mechanism of hernia-associated fibrosis and provides valuable insights into the broader field of fibrotic diseases and points toward potential therapies.

## SIGNIFICANCE/CLINICAL RELEVANCE:

Our study highlights the critical involvement of E2/ESR1 signaling which drives fibrosis in inguinal hernias, offering insights into the potential reversibility of fibrosis and suggesting novel pharmacological avenues for therapeutic intervention.

## REFERENCES:

[1] Zhao *et al.* "Shift from androgen to estrogen action causes abdominal muscle fibrosis, atrophy, and inguinal hernia in a transgenic male mouse model." *Proceedings of the National Academy of Sciences* 115.44 (2018): E10427-E10436. [2] Potluri *et al.* "An estrogen-sensitive fibroblast population drives abdominal muscle fibrosis in an inguinal hernia mouse model." *Journal of Clinical Investigation Insight* 7.9 (2022).

