PADI4-Mediated Chromatin Externalization in Senescent Fibroblasts to Sustain Chronic Inflammatory Microenvironment

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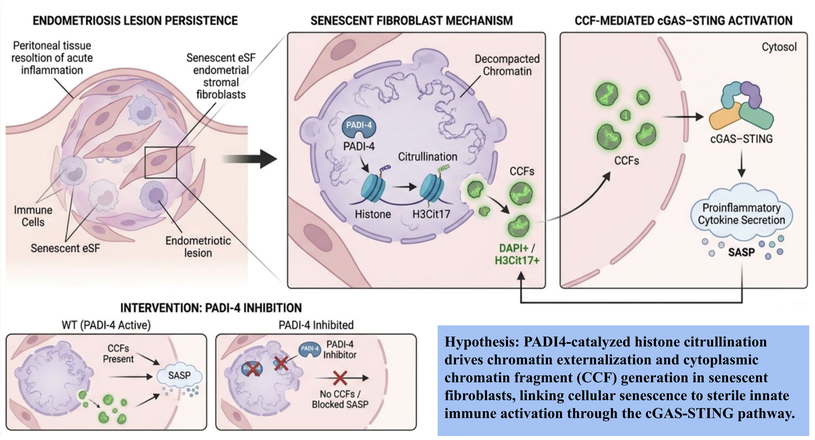
GRAPHICAL ABSTRACT



Hypothesis: PADI4-catalyzed histone citrullination drives chromatin externalization and cytoplasmic chromatin fragment (CCF) generation in senescent fibroblasts, linking cellular senescence to sterile innate immune activation through the cGAS-STING pathway.

INTRODUCTION

Endometriosis Pathology & Lesion Persistence

- Retrograde menstruation deposits endometrial fragments into the peritoneal cavity frequently during menstruation, yet only approximately 10% of women develop endometriosis. The presence of ectopic tissue is therefore insufficient to explain disease; lesion establishment also requires failed immune clearance by immediate neutrophils and fibroblasts, which end up adding to the lesion
- Early neutrophils are short-lived at the lesion, however, NET formation alone cannot explain how this inflammatory niche is maintained after the initial lesion-seeding phase. A longer-lived stromal population may be required to sustain the lesion. Endometrial stromal fibroblasts (eSFs) are plausible lesion-maintenance cells because the early neutrophilic response can both recruit them and induce their senescence.
- One mechanism linking nuclear damage to the SASP is the formation of cytoplasmic chromatin fragments (CCFs). During senescence, loss of lamin B1 and disruption of the nuclear lamina allow chromatin to bud from the interphase nucleus into the cytoplasm, activating the cGAS-STING pathway which can recruit inflammatory cytokines to tissue fibrosis/vascularization.

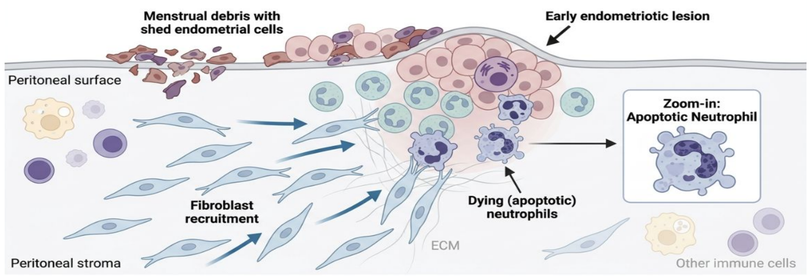


Diagram depicting early endometriotic lesion on the peritoneal surface with dying neutrophils (apoptotic), and the recruitment of spindle-shaped fibroblasts toward the lesion area. Other immune cells are illustrated for microenvironment context.

Role of PADI4-Mediated Citrullination

- PADI4-driven histone citrullination in senescence: catalyzes citrullination of histone H3 (particularly H3Cit17), destabilizing chromatin and promoting nuclear envelope breakdown, a hallmark of senescent cell progression
- Cytoplasmic chromatin fragments (CCFs) as danger-associated molecular patterns: PADI4-induced citrullination releases fragmented DNA into cytoplasm, activating cGAS-STING pathway, an immune sensor amplifying pro-inflammatory and pro-fibrotic transcription
- cGAS-STING activation drives both inflammation and fibrosis: triggers type I interferon responses and NF-κB signaling, sustaining IL-6/TNF-α production, enhancing TGF-β signaling for fibrotic remodeling, and preventing senescent cell clearance
- PADI4 in disease amplification: links senescence establishment to chromatin extrusion and innate immunity, converting senescence from protective to persistent inflammatory pro-fibrotic state sustaining endometriosis lesion biology

DATA COLLECTION

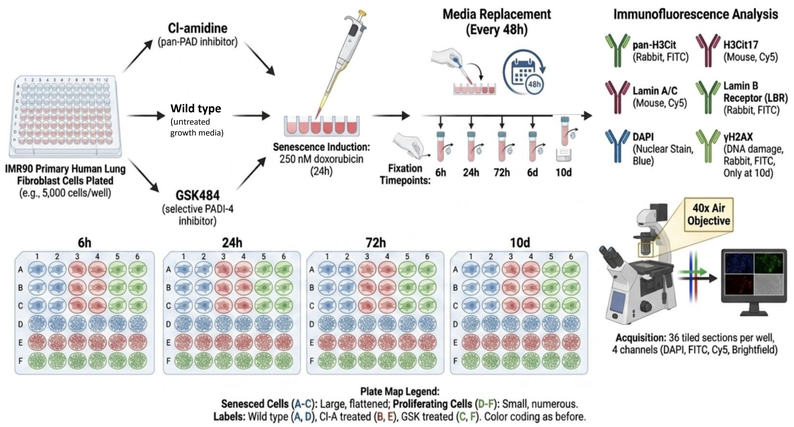
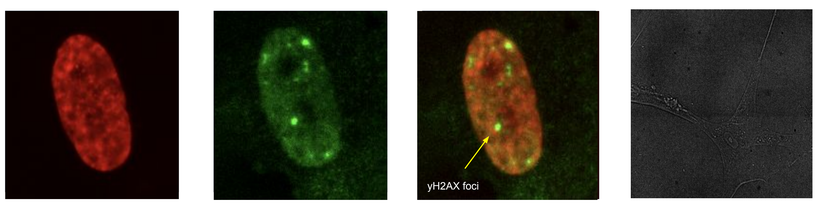


Plate Map Legend:
Senesced Cells (A-C): Large, flattened; **Proliferating Cells** (D-F): Small, numerous.
Labels: Wild type (A, D), Cl-A treated (B, E), GSK treated (C, F). Color coding as before.

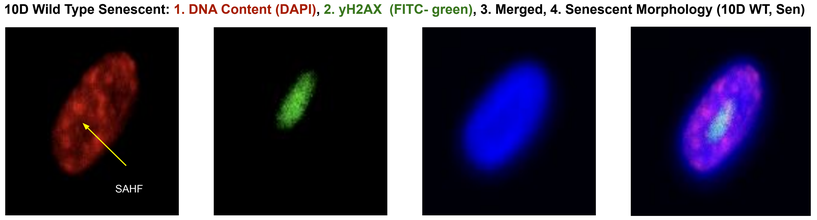
RESULTS

- **PADI4 activity precedes and facilitates nuclear envelope disruption**: Lamin B receptor (LBR) loss and Lamin A/C redistribution from the nuclear periphery become prominent by 72HR–6D in senescent controls. Blebbing of the nuclear membrane with inconsistent Lamin A/C and LBR patterning was only seen in wild type senescent cells after 72HR, likely as a CCF precursor.
- **PADI4-dependent chromatin extrusion generates cytoplasmic chromatin fragments (CCFs)**: By 10 days, extensive γH2AX-positive extranuclear chromatin structures accumulate in senescent wild type populations. CCFs were γH2AX-positive. PADI4 inhibition significantly reduces CCF frequency and extent, indicating sustained PADI4 activity throughout senescence is required for efficient chromatin extrusion
- **PADI4-mediated CCF formation is senescence-specific**: CCF formation and PADI4-dependent effects occur exclusively in senescent populations, not in proliferating controls, indicating the process is coupled to senescence-associated changes in chromatin and nuclear organization rather than constitutive PADI4 activity.
- **PADI4 is the dominant PAD isoform in this pathway**: Selective PADI4 inhibition (GSK484) produces effects comparable to broad PAD inhibition (Cl-amidine) across all time points and readouts, establishing PADI4 as the primary driver of histone citrullination and nuclear destabilization during fibroblast senescence. This is also established by the analogous patterns of H3Cit17 and pan-H3Cit activity, as H3Cit17 is a PADI-4-specific citrullination epitope.
- **CCF-extruding cells lose chromatin density:** Cl-A senescent treated cells showed highly dense chromatin regions (Senescence Associated Heterochromatin Foci) even at 10D, a senescence hallmark. These foci were not clearly present in cells actively externalizing CCFs.

CHROMATIN CONDENSATION/DNA DAMAGE IMAGING

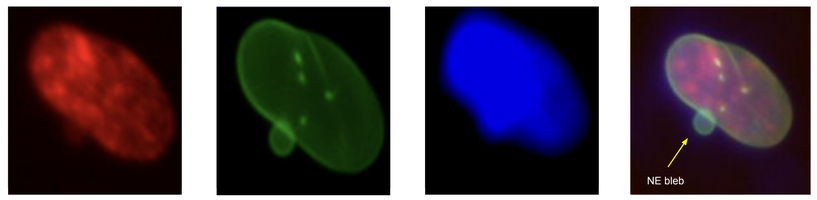


10D Wild Type Senescent: 1. DNA Content (DAPI), 2. γH2AX (FITC- green), 3. Merged, 4. Senescent Morphology (10D WT, Sen)

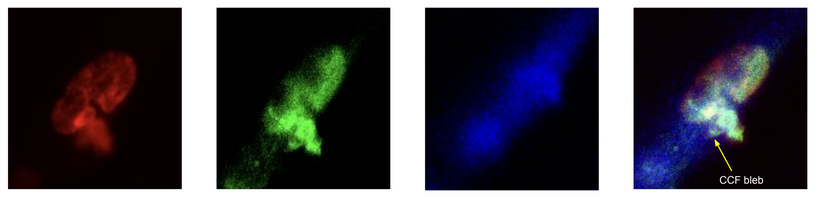


72HR Cl-A Type Senescent: 1. DNA Content (DAPI), 2. H3Cit17 (FITC- green), 3. Lamin A/C (Cy5- blue), 4. Merged

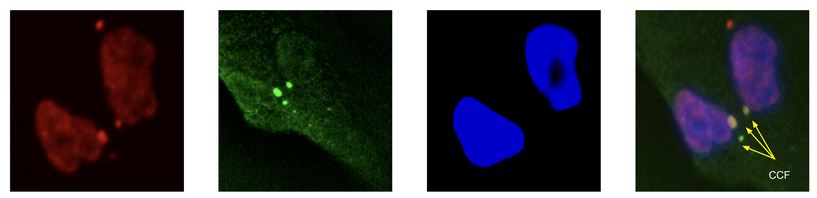
CYTOPLASMIC CHROMATIN FRAGMENT IMAGING



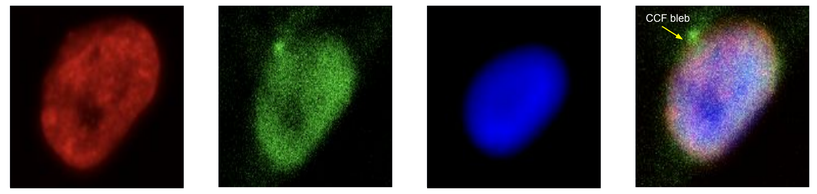
72HR Wild Type Senescent: 1. DNA Content (DAPI), 2. H3Cit17 (FITC- green), 3. Lamin A/C content (Cy5- blue), 4. Merged



10D Wild Type Senescent: 1. DNA Content (DAPI), 2. H3Cit17 (FITC- green), 3. Lamin A/C content (Cy5- blue), 4. Merged



10D Wild Type Senescent: 1. DNA Content (DAPI), 2. H3Cit17 (FITC- green), 3. LBR (Cy5- blue), 4. Merged



10D Wild Type Senescent: 1. DNA Content (DAPI), 2. H3Cit17 (FITC- green), 3. LBR (Cy5- blue), 4. Merged

CONCLUSION

- **PADI4 as persistence mechanism**: Establishes PADI4-catalyzed chromatin modification as critical molecular node linking senescent fibroblast establishment to CCF generation by citrullination and decondensation of chromatin for extrusion via the disrupted NE
- **Therapeutic window for intervention**: Sustained PADI4 activity throughout senescence time course suggest PADI4 is attractive therapeutic target for disrupting senescence-to-persistence transition in endometriotic lesions, with potential to block both initial senescence amplification and ongoing inflammatory consequences, giving further evidence for early senomorphic treatments
- **Senescence as amplification mechanism**: Demonstrates PADI4 converts senescent fibroblasts from proliferation-arrest state into active generators of damage signals (CCFs, γH2AX foci, NE blebs)
- **Proposed model for endometriosis**: Endometrial fibroblasts within lesions, exposed to chronic inflammation and oxidative stress, undergo senescence as protective response. However, PADI4 activity during senescence-establishment catalyzes histone citrullination, nuclear envelope compromise, and CCF extrusion, triggering cGAS-STING-mediated immunity that sustains lesion inflammation and fibrosis

KEY CITATIONS

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