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Senescent Synoviocytes in Specific Regions of Osteoarthritic Knees Correlate With Disease, Biomarkers, Synovitis, and Knee Pain

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Introduction

- Cellular senescence is a natural state in which a cell permanently halts division through upregulation of a set of intracellular proteins, including $p16^{INK4A}$ (p16), in response to various cellular stressors[1]
- Senescent cells secrete factors collectively referred to as the Senescence Associated Secretory Phenotype (SASP) that are proinflammatory and degradative in the local tissue environment[2]
- With aging, senescent cells accumulate in multiple tissues,[3] and senolytic therapies are being developed to address age-related diseases including osteoarthritis (OA)[4] (Poster L05, ACR 2019)
- OA is characterized by the presence of inflammatory and degradative factors that suggests a role for senescence in its pathobiology; however the characterization of the relationship between senescence burden, synovitis, and pain in OA is complicated by the heterogenous nature of the disease
  - It has been shown, for example, that certain synovitis patterns are better predictors of pain[5]
- Therefore, we conducted a study to explore the relationship between the regional burden of senescence and synovitis, pain, and synovial fluid SASP factors of the knee

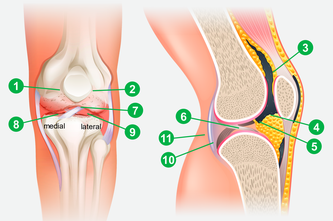
Objectives

- **Primary:** to assess the relationship between degree of senescence burden in synovial tissue as defined by the percentage of cells staining positive for p16 by immunohistochemistry (IHC) analysis and candidate biomarkers of senescence in patients with OA of the knee
- **Additional:** to determine the relationship between the degree of synovial senescence and synovitis, OA severity and knee pain

Methods

- Study design: non-drug, cross-sectional, single-center study
- Assessments: Kellgren-Lawrence (KL) grading by plain radiography; synovitis score by gadolinium enhanced magnetic resonance imaging (GE-MRI),[6] Western Ontario and McMaster Universities OA Index (WOMAC-A) pain subscale
- Key eligibility criteria included age ≥35 years, diagnosis of primary idiopathic femoro-tibial OA of the knee, KL grade 1–4 and body mass index (BMI) ≤35 kg/m^2
- Samples of synovial fluid and shave biopsies of synovium were obtained during arthroscopy and the latter analyzed for the presence of p16 via IHC analysis
  - Sectioned tissue was stained for p16 expression by IHC using CINtec® antibody (clone E6H4™, Roche, Switzerland) on a Leica Bond instrument; the degree of senescence was quantified in these samples by counting the number of p16$^+$ cells and the total cell number in the synovium; Visiopharm (Denmark) software was used for identification and quantification
  - Putative biomarkers were measured in synovial fluid by bead-based enzyme-linked immunosorbent assay (ELISA; Luminex, Austin, TX) and mass spectrometry proteomics (MS-SWATH)
  - Percent p16$^+$ cells was calculated as the total number of p16$^+$ cells divided by the total cell number in specific regions for a patient; correlations between clinical or biomarker variables were calculated using partial Spearman's rank correlation adjusting for age and BMI as well as Spearman's rank correlation without covariate adjustment; analysis of covariance (ANCOVA) was also performed to assess the effect of a variable on another while adjusting for age and BMI

Knee Regions Assessed for Synovitis Severity Using a Semi-Quantitative Scoring System With GE-MRI



Region	Anatomical Location
1	Medial parapatellar recess
2	Lateral parapatellar recess
3	Suprapatellar
4	Infrapatellar
5	Intercondylar
6	Adjacent to PCL
7	Adjacent to ACL
8	Medial perimeniscal
9	Lateral perimeniscal
10	Adjacent to loose bodies
11	Bakers cyst

ACL, anterior cruciate ligament; PCL, posterior cruciate ligament.

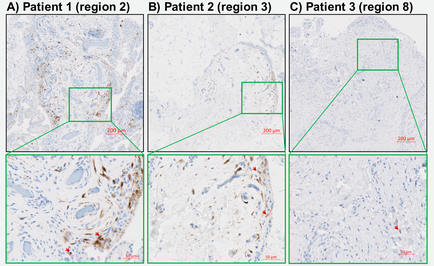
Results

Demographics

Demographic Characteristic	Total N=30
Age, mean years (range)	59.4 (43–77)
BMI, mean kg/m^2 (range)	29.7 (24.3–34.9)
Sex, n (%)	
Male	16 (53.33)
Female	14 (46.67)
Ethnicity, n (%)	
Hispanic or Latino	0
Not Hispanic or Latino	30 (100)
Race, n (%)	
Black or African-American	7 (23.33)
White	23 (76.67)
KL grade, n (%)	
1	2 (6.67)
2	13 (43.33)
3	7 (23.33)
4	8 (26.67)

- The study population was typical for the knee OA patient population in the US in terms of demographics and KL grades

Representative IHC Images From 3 Anatomical Regions In Different Patients

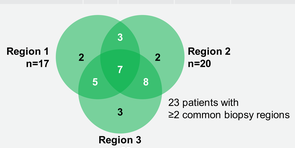


IHC staining for p16 in synovium shave biopsies (brown); slides are counterstained with hematoxylin (blue). Zoomed regions are outlined in green. Red arrows point to examples of p16$^+$ cells.

- Two biopsies (A & B) from regions 2 and 3 show high senescence burden (>5% of cells are p16$^+$), while one (C) from region 8 has a low senescence burden (<1% of cells are p16$^+$)

Number of Patients, Total and p16$^+$ Counted Cells and Synovitis Score Per Biopsy

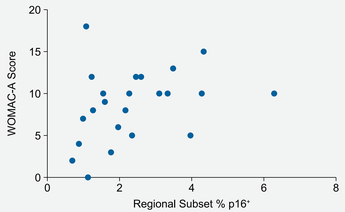
Region	Anatomical Location	n	Median no. cells	Median no. p16$^+$	Mean Synovitis score
1	Medial parapatellar recess	17	7,191	119	1.18
2	Lateral parapatellar recess	20	8,892	132.5	1.25
3	Suprapatellar	23	10,708	168	1.35
5	Intercondylar	15	3,635	29	0.93
7	Adjacent to ACL	11	10,232	171	1.27
8	Medial perimeniscal	27	7,666	63	0.48
9	Lateral perimeniscal	7	5,281	37	1.00



n=number of patients with biopsy taken; # cell=total number of cells per biopsy; # p16$^+$=number of p16$^+$ cells per biopsy; synovitis score=GE-MRI synovitis score of biopsied regions. No biopsies were taken from regions 4, 6, 10 and 11.

- Regions 1, 2 and 3 meet a consistent threshold for number of patients, total cell number and total p16$^+$ cells
- Further analyses focused on % p16$^+$ cells from these 3 regions in patients where at least 2 of those 3 regions were biopsied; 23 of 30 patients met this criteria

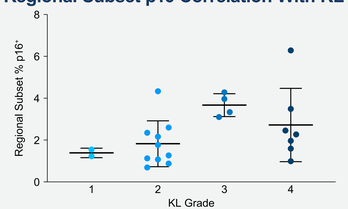
Regional Subset p16 Correlation With WOMAC-A



Partial Spearman's rank correlation: 0.471, p=0.042, n=23. Data adjusted for age and BMI. Regions 1, 2 and 3 analyzed.

- Percent of p16$^+$ cells in regions 1, 2 and 3 synovial biopsies correlates positively with pain as measured by the WOMAC-A pain subscale

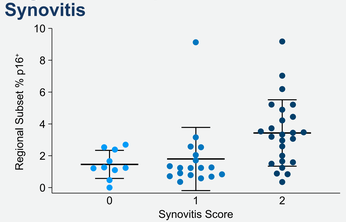
Regional Subset p16 Correlation With KL



ANCOVA of % p16$^+$. KL 1–4: p=0.156; 1–3: p=0.0317; 2–3: p=0.0305. Data adjusted for age and BMI. Regions 1, 2 and 3 analyzed. Mean ± standard deviation (SD) shown.

- Percent of p16$^+$ cells in regions 1, 2 and 3 synovial biopsies increases as KL grades increase from 1 to 3

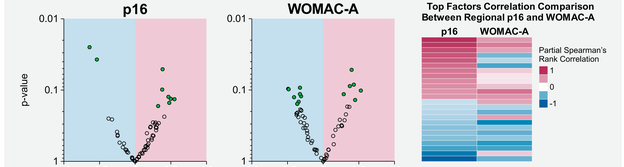
Regional Subset p16 Correlation With Synovitis



ANCOVA of % p16$^+$. Synovitis 0–2: p=0.0298. Data adjusted for age and BMI. Regions 1, 2 and 3 analyzed. Mean ± SD shown.

- Percent of p16$^+$ cells in regions 1, 2 and 3 synovial biopsies increases with degree of synovitis

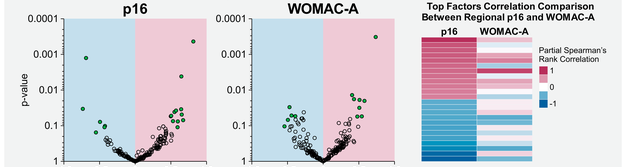
Regional p16 and WOMAC-A Correlation With Synovial Fluid Biomarkers (Luminex panel)



Partial Spearman's rank correlation displayed. Data adjusted for age and BMI. Regions 1, 2 and 3 analyzed. 61 protein factors analyzed by Luminex ELISA in the synovial fluid of 23 and 30 patients for p16 and WOMAC-A, respectively. Green circles indicate candidate factors.

- 9 and 14 biomarker candidates are found for p16 and pain, respectively
- Of the top 24 factors correlating with tissue p16$^+$ cell burden (positive and negative), 18 share a similar direction of correlation with WOMAC-A pain subscale

Regional p16 and WOMAC-A Correlation With Synovial Fluid Biomarkers (Proteomics)



Partial Spearman's rank correlation displayed. Data adjusted for age and BMI. Regions 1, 2 and 3 analyzed. 191 protein factors identified by mass spectrometry in the synovial fluid of 23 and 30 patients for p16 and WOMAC-A, respectively. Green circles indicate candidate factors.

- 17 and 14 biomarker candidates are found for p16 and pain, respectively
- Of the top 24 factors correlating with tissue p16$^+$ cell burden (positive and negative), 14 share a similar direction of correlation with WOMAC-A pain subscale

Conclusions

- p16$^+$ cells are found in the synovial tissue of knee OA patients
- The level of p16$^+$ cells in selected regions of the synovium correlates with clinical markers of pain, tissue degradation and synovitis
- Several synovial fluid biomarkers have been identified based on their correlation with p16 burden in specific synovial tissue regions and with pain
- Together these results provide a strong rationale for senolytic therapies for OA of the knee

References: **1.** Zindy F, et al. Oncogene 1997;15:203-11; **2.** Coppé J-P, et al. PLoS Biology 2006;6:2853-68; **3.** Baker DJ, et al. Nature 2016;530:184-9; **4.** Childs BG, et al. Nature Reviews Drug Discovery 2017;16:718-35; **5.** de Lange-Brokaar BJE, et al. Arthritis & Rheumatology 2015;67:733-40; **6.** Guermazi A, et al. Ann Rheum Dis. 2011;70:805-11.

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