



The Analysis of Senescent Gene Expression in Human Gingival Tissue Affected by Periodontitis



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Background

Accumulation of senescent cells are increasingly recognized as a fundamental mechanism of aging and are regarded as an attractive therapeutic target for various age-associated pathological conditions. Although the prevalence and severity of periodontitis are highly associated with age, and preclinical studies have demonstrated that senescent cells could be a potential therapeutic target of age-associated alveolar bone loss, clinical data that indicates the association between cellular senescence and periodontitis is limited.

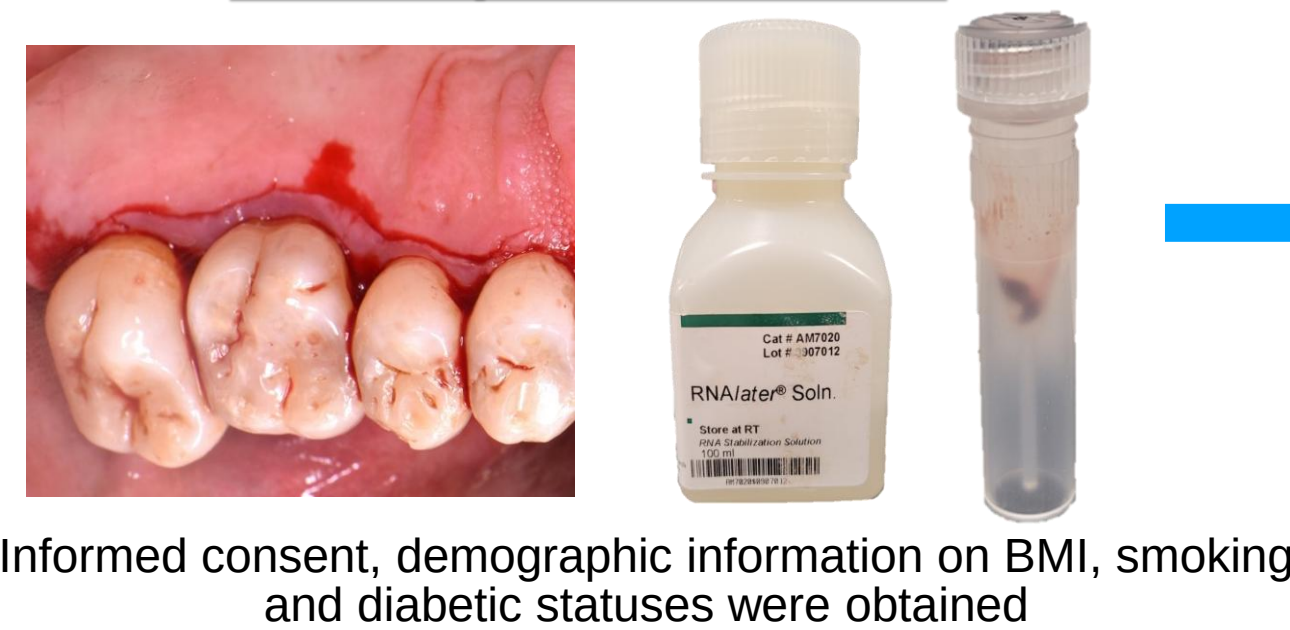
Purpose

The purpose of this cross-sectional study was to compare the level of senescence markers in periodontitis and non-periodontitis gingival tissues

Methods

Gingival tissues were collected from patients (N=54) with or without periodontitis. Gene expression analysis was performed for senescence-associated genes, including cell cycle inhibitors (*p16*, *p21*, *p15*, *TP53*), anti-apoptotic genes (*BCL2*, *BCL2XL*), and senescence-associated secretory phenotype (SASP) genes (*IL1B*, *IL6*, *IL17A*, *IL8*, *MMP2*, *MMP8*, *MMP12*, *MMP13*, *MMP14*). Chi-square tests were used to compare patient characteristics between the groups with or without periodontitis. Comparisons between the groups for differences in senescence marker expression were analyzed using two-sample *t*-tests with heterogeneous variances.

1. Sample collection



Informed consent, demographic information on BMI, smoking and diabetic statuses were obtained

2. RNA Isolation from gingival samples



- Lyse samples with Bashing Beads
- Homogenize samples in TRIzol
- Phase separation
- Precipitate RNA
- Wash and solubilize RNA
- Determine RNA yield by absorbance

4. qPCR Reaction

Primers were designed with NIH primer-blast

3. Synthesis of cDNA

Reverse transcription using high-capacity RT kit

Inclusion criteria:

- ✓ Patients receiving periodontal surgery (e.g. osseous surgery, crown lengthening)
- ✓ ≥18 years old
- ✓ ASA class I or II

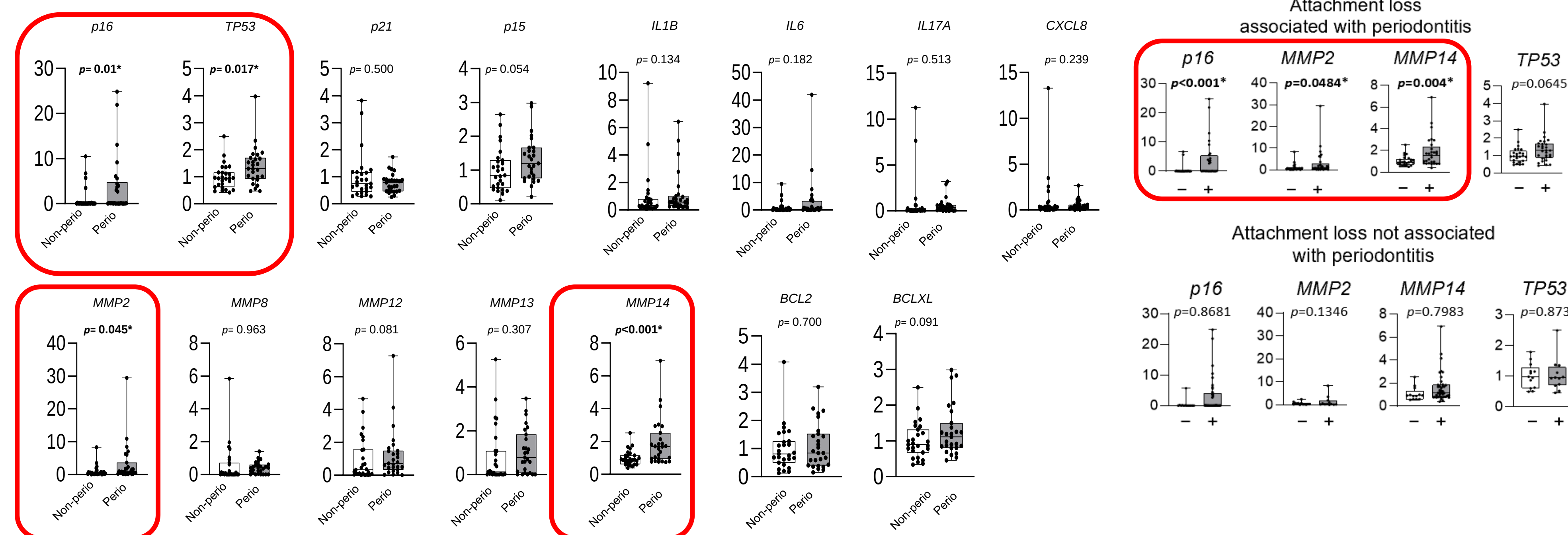
Exclusion criteria:

- ✓ Pregnant or nursing women
- ✓ Presence of active dental infection at the time of surgery

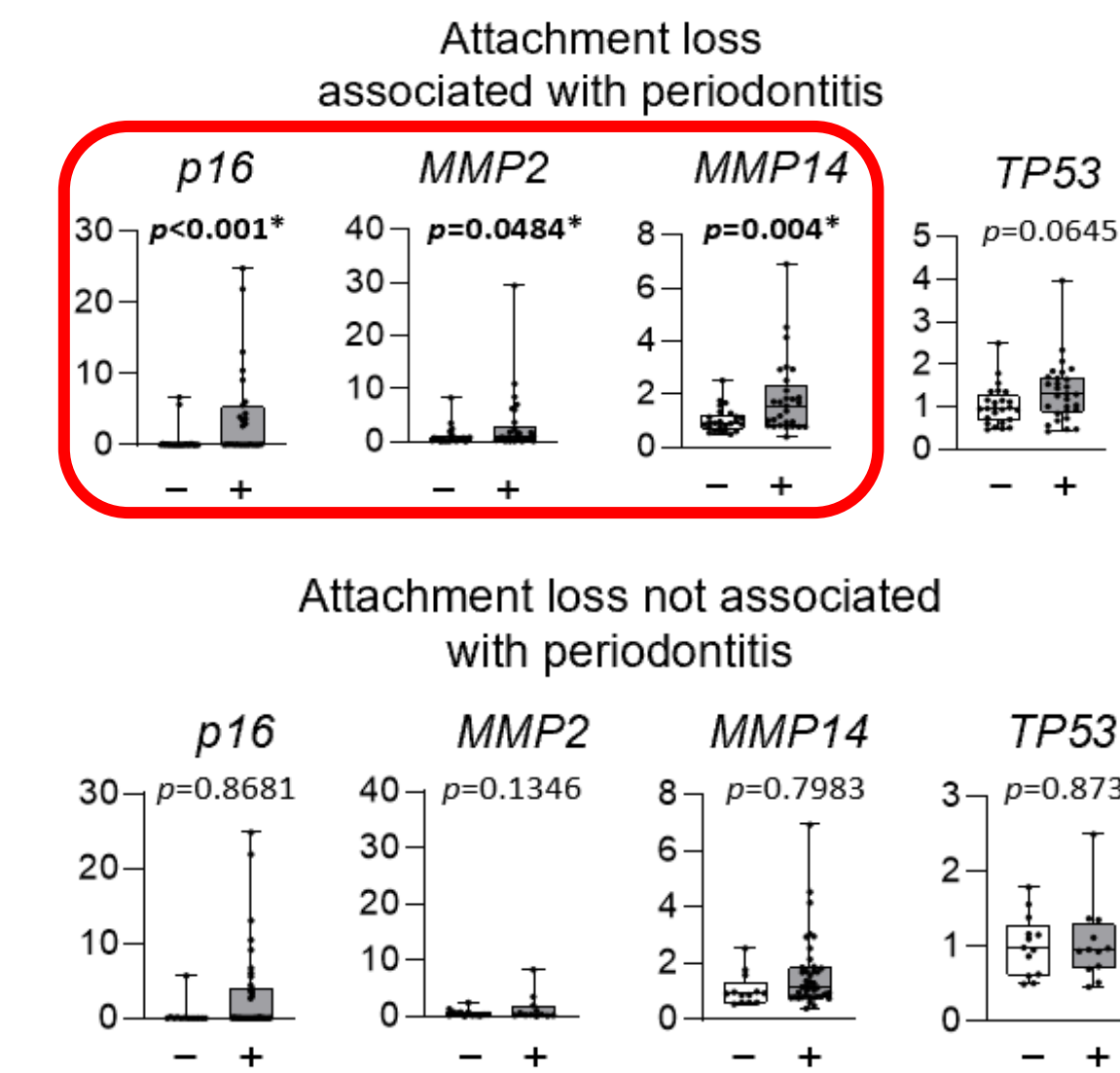
Results

Total of 54 gingival tissue samples were included in the study. Of the 54 samples, 27 were from patients diagnosed with periodontitis while the other 27 were from patients without periodontitis. The mean ages of non-periodontitis group and periodontitis group were 48.3 years old (SD 17.3) and 53.8 years old (SD 14.7), respectively. Among the genes analyzed, *p16*, *TP53*, *MMP2*, and *MMP14* showed significantly higher expression in the group diagnosed with periodontitis than control group ($p = 0.010$, 0.017 , 0.045 , and <0.001). Additionally, these genes were associated with the clinical attachment loss due to periodontitis. The expression levels of inflammatory genes did not differ significantly between the periodontitis group and the control group. There were no statistically significant positive correlations between the gene expression levels and age.

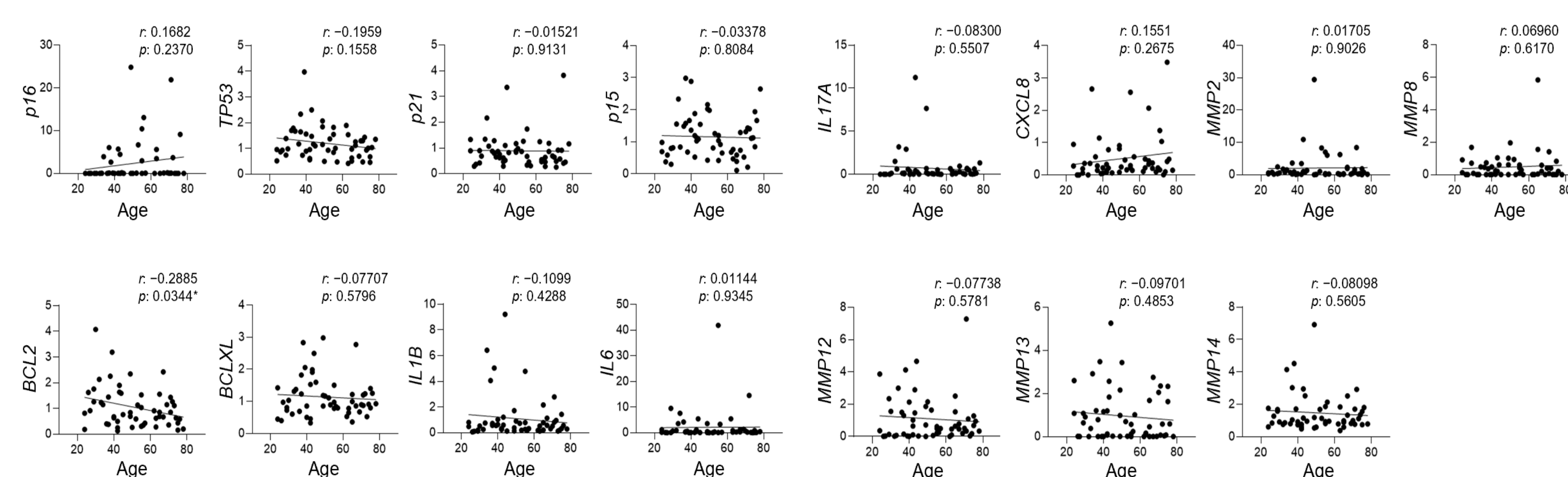
Senescence-associated gene expression



Association with attachment loss



Association with age and other factors



Discussion

The expression levels of *p16*, *MMP2* and *MMP14* were highly associated with the gingival inflammatory status and attachment loss caused by periodontitis, which aligns with the concept that senescent cell accumulation in periodontal tissue drives periodontal inflammation and tissue damage. Cellular senescence might be an induction mechanism of MMPs during periodontitis. Also, the upregulated *TP53* in gingival tissues from periodontitis patients may be involved in regulating host immune responses rather than contributing cellular senescence. None of the tested genes positively correlated with age in human gingival tissue, suggesting that chronological aging alone is not sufficient to increase senescent burden in human gingival tissue. Instead, being affected by periodontitis seems to increase senescent burden in periodontal tissue. In the present study, cell types that were responsible for the senescent characteristics in the gingival tissue affected by periodontitis were unable to be identified. Also, the functional impact of cellular senescence on the pathology of periodontitis was not able to be assessed.

Conclusion

In conclusion, gene expression analysis indicates an association between periodontitis and senescent cell accumulation in periodontal tissue and upregulation of *p16*, *TP53*, *MMP2* and *MMP14* would be a characteristic of periodontitis-associated senescence. Further research is needed to help understand the causal relationship of senescence and periodontitis, as well as the impact of cellular senescence on the pathogenesis of periodontitis.

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