Epigenetic characteristics in inflammatory candidate genes in aggressive periodontitis: The role of interleukin 17C and CCL25

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Introduction

Periodontitis is a complex inflammatory disease

- Exogenous risk factors
  - Medical/dental plaque formation
  - Tobacco smoking
  - Endogenous risk factors
  - Genetic predisposition
  - Immune response
  - Epigenetic modifications

Periodontitis

DNA methylation
Histone modifications
Chromatin remodeling
DNA-methylation is involved in transcriptional regulation
leads to gene silencing

Material and Methods

Patients and controls

- Preliminary case-control study
  - All patients and healthy controls were of Caucasian descent
  - Patients with aggressive periodontitis
    - n = 21
    - clinical manifestation < 30 years
    - Clinical Attachmentloss > 4mm in 30% of teeth
  - Periodontitis-free controls
    - n = 25
    - age ≥ 30 years
    - Clinical Attachmentloss ≤ 3.5mm

Exclusion criteria for all participants:
- periodontal treatment during the last 6 months,
- antibiotic therapy during the last 3 months,
- pregnancy,
- Occurrence of systemic diseases

Genomic investigations

- Sample preparation: gingival biopsies
- During dental surgery gingival biopsies were obtained from inflamed tissue from patients with aggressive periodontitis and from periodontally healthy controls

DNA-Isolation from gingival biopsies

Preparation of genomic DNA was carried out using the QiAamp® DNA Micro Kit (Qiagen, Hilden, Germany).

Epigenetic methylation pattern

- DNA samples were cleaved using EpiTect® Methyl DNA restriction Kit (Qiagen, Hilden Germany)
- 1: no enzyme
- 2: methylation sensitive enzyme
- 3: enzyme that is not sensitive for methylation
- 4: both enzymes

- For analysing CpG methylation pattern EpiTect® Methyl II Signature PCR Array Human Inflammatory response (Qiagen, Hilden Germany) was applied

- Real-Time-PCR was carried out using SYBR® Green (RT-SYBR Green ROX qPCR Mastermix, Qiagen, Hilden, Germany)

- PCR-System: Applied Biosystems 7500 Real-Time PCR System

PCR-Program:
1 cycle: 93°C, 10min, hot start for activation of DNA polymerase
3 cycles: 99°C, 30sec, 72°C, 1min
40 cycles: 97°C, 15sec, 72°C, 1min, detection of SYBR®-green fluorescence in every cycle, end of reaction

Results and discussion

Clinical characterization of the patient groups

<table>
<thead>
<tr>
<th>Genesymbol</th>
<th>Patients with aggressive Periodontitis</th>
<th>Periodontitisfree Controls</th>
<th>p values</th>
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<tr>
<td></td>
<td>n=11</td>
<td>n=25</td>
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<td>CCL25</td>
<td>17.4±4.4</td>
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<td>CCL14</td>
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<td>CCL3</td>
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<td>CCL5</td>
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<td>IFN2</td>
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<td>GATA3</td>
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<td>8.3±15.5</td>
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<td>IL12A</td>
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<td>IL12B</td>
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<td>IL13</td>
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<td>IL13R1A1</td>
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<td>IL15</td>
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<td>IL17</td>
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<td>26.4±22.0</td>
<td>0.007*</td>
</tr>
</tbody>
</table>

* Student's T Test

Epigenetic - Cytokines - Periodontitis

- Cytokines are inducers of alveolar bone loss and collagen degradation
- Cytokines promote inflammatory processes of periodontitis
- Epigenetic pattern can influence cytokine expression

Hypotheses and aims of the present study:

- Because of the possible epigenetic control of cytokine expression and its potential role in periodontitis manifestation an development we investigated the CpG methylation pattern of 22 inflammatory candidate genes (APC2, CCL25, CXCL4, CXCL3, CXCL5, CXCL6, FADD, GATA3, IL10RA, IL12A, IL12B, IL1, IL13R1A1, IL13, IL17C, IL17RA, IL4R, IL6R, IL10R, IL10, TYK2) in dependence of periodontal status.

Epigenetic evaluation

- In gingival inflamed tissue of patients with aggressive periodontitis there was a significant reduction in CpG methylation pattern of CCL25 and interleukin 17C compared with tissue of periodontal healthy persons.

Conclusions

In this preliminary study we showed for the first time a differential methylation pattern for CC125 and IL17C in periodontitis. CCL25 is involved in T-cell development and IL17C plays a role in epithelial immune response induced by bacterial challenge and inflammatory stimuli. The decrease in methylation is presumably accompanied by an increase in gene expression. This could result in a greater availability of CCL25 and IL17C and the induction and progression of periodontal inflammation.