Introduction

Periapical periodontitis is mainly caused by bacterial infection within the root canal, and periapical tissue destruction is caused by endogenous inflammatory mediators released during interaction between bacteria and host cells, mainly polymorphonuclear neutrophils, lymphocytes, plasma cells, and macrophages. However, the pathological mechanisms that are responsible for apical tissue destruction such as bone degradation have not been fully understood.

 Clinically, judgment of the success of root canal treatment (RCT) is abundant but inconclusive. In many clinical cases, the success has been evaluated by radiographic healing of periapical lesions. Also, it might be evaluated by decrease of clinical symptoms such as spontaneous pain, percussion pain and pus discharge. Considering the inflammatory responses in periapical lesions, the judgment of the success should be evaluated by decrease of inflammatory reactions. However, molecular mechanisms regulating inflammatory process, especially healing process after RCT in periapical lesions are still poorly understood.

Comprehensive gene expression analysis with cDNA microarray is a powerful tool to investigate the etiology and pathophysiology of diseases including periapical lesions. The altered gene expression pattern detected by cDNA microarray analysis suggests that complex inflammatory reactions during RCT are likely to be involved in development and consequence of the disease. In the present study, we established in vivo model of the periapical lesions with RCT to clarify the molecular pathophysiology of the disease. Using cDNA microarray analysis, we report for the first time a global gene expression analysis in periapical lesions with RCT.

Materials and Methods

Experimental animal models of periapical lesions during RCT

Periapical lesions were induced experimentally by exposing the dental pulp of Sprague-Dawley rats (male, 10 w). After 3 weeks of pulp exposure, the animals (4 rats) received root canal filling (RCF), and were sacrificed 1 or 4 weeks later. From the periapical tissues, total RNA was extracted (3 rats). The lesions were histologically (1 rat) and radiographically (4 rats) confirmed to expand 4 weeks after pulp exposure (inflammation phase) and to stabilize 4 weeks after RCF (healing phase).

Microarray analysis and Quantitative RT-PCR
Rat genome 230 2.0 arrays, which contained approximately 30,000 probes corresponding to known rat genes, were purchased from Affymetrix (Santa Clara, CA, USA). For cDNA microarray analysis, a series of reverse transcription, second-strand cDNA synthesis, and probe generation were accomplished and processed according to manufacturer's instructions (Affymetrix). Scanned images were analyzed using GeneChip Operating Software (Version 1.1: Affymetrix) and GeneSpring software (version 7.2: Tomy Digital Biology, Tokyo, Japan). The genes showing greater than a fold-change above 5.0 or below -5.0 were selected as expression-changed genes. Pathway analysis was performed using Ingenuity Pathway Analysis software (www.ingenuity.com, Ingenuity® Systems, Redwood City, CA, USA). The quantitative (q) RT-PCR was performed using Chromo 4 Real-time PCR Detector (Bio-Rad, Hercules, CA, USA). From microarray data, IL-1β, caspase 8, cathepsin L, IL-1α, and defensin α5 were selected.

**Immunohistochemistry**

Immunohistochemistry was performed by an indirect peroxidase technique. Primary antibodies used in this study were rat anti-IL-1α antibody and rat anti-IL-1β antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The staining without the primary antibody was used as negative control.

**Results**

1. In approximately 30,000 genes on the microarray, 203 genes were up-regulated by over 5-fold (IL-1β, etc.) and 864 genes were down-regulated to less than 20% of baseline level (caspase-8, etc.) in inflammation phase. Compared to inflammation phase, we found that 133 genes were up-regulated (IL-1α, etc.) and 50 genes were down-regulated (defensin-α5, etc.) in healing phase.

2. Besides caspase 8 gene, it was demonstrated that gene expression patterns correlated, but generally higher fold-changes were observed in qRT-PCR compared to those obtained in the microarray experiments.

3. The pathway analysis revealed that interactions of genes during RCT are complex as expected. Especially, we found that increase of IL-1α gene is an important factor in healing phase following inflammation phase. Also, tumor necrosis factor (TNF) gene and transforming growth factor (TGF) β1 gene were suggested to play a central role in healing phase, although the expression of their genes did not show obvious change during RCT.

4. Corresponding to the gene expression profiles, accumulation of IL-1α and IL-1β was observed in the periapical lesions by immunohistochemistry.

**Discussion and Conclusion**

cDNA microarray analysis of global gene expression patterns provided new molecular candidates for the diagnosis of periapical healing after RCT using in vivo models. Our findings suggest biological significance of differential gene expression and confirm some results in the literature with regard to molecules known to be involved in the periapical healing. These genes provide a basis for further studies of periapical healing after RCT, and might be useful as attractive targets for diagnosis.
論文審査結果の要旨

本論文は、専門分野である歯内療法の先導的専門誌である米国歯内療法学会誌（Journal of Endodontics）に掲載されることになっているものであった。

本研究は、動物実験モデルを用いて、感染根管治療後の根尖部に見られる組織の治癒過程を、遺伝子マイクロアレイ解析とバイオインフォマティクスの手法を用いて検討したものである。とりわけ、非常に繊細なテクニックを必要とするラット臼歯の根管充填を成功裏に行った点は、本研究結果を見出すために非常に重要なポイントである。また、組織学的およびX線的な検討によって、構築した感染根管治療モデルの妥当性を証明した点は、本研究の意義を高めるものである。総じて、世界で初めて感染根管治療の各ステージを動物実験モデルに再現し得た研究デザインは非常に価値があると評価する。

得られた結果は、根尖病巣の治癒の病態を分子生物学的に網羅的に捉えており、将来、根尖性歯周炎に対する治療の成否を、分子レベルで診断するための候補遺伝子を複数得ることができた点は、今後の本領域の研究レベルの発展に大きく貢献するものである。

また申請者は、本研究を新規的なものにするため、遺伝子マイクロアレイ解析のノウハウを有する日本大学松戸歯学部生化学講座との共同研究に発展させ、価値ある結果を得た。その研究に対する姿勢は高く評価し得る。

また、本論文の内容は、2007年3月に開催された第85回国際歯科学研究学会（IADR）においてポスター発表として公開され、Pulp Biology Regeneration GroupからStudent Travel Awardを受賞したことを確認した。