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Tannerella Forsythia in Oral Squamous Cell Carcinoma - An Exploratory Study

Research Article

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Abstract

Background: While no single species has been implicated as the primary pathogen and the available evidence is consistent with a polymicrobial disease etiology, the red-complex bacteria consisting of Porphyromonas gingivalis, Treponema denticola and *Tannerella forsythia* has been strongly implicated in the onset of periodontitis a chronic inflammatory disease affecting the supporting tissues of the teeth in the oral cavity. Chronic inflammation is believed to act as catalyst for tumour development and progression.
Objective: To study the presence of *Tannerella forsythia* in the tissue samples oforal squamous cell carcinoma.
Materials and Methods: Oral squamous cell carcinoma (n= 30) and oral mucosal non-cancerous tissue specimens (n=30) were obtained from patients and controls separately. RNA was isolated from each aseptically deliquesced tissue specimen and transformed to cDNA using the Trizol technique. Specific gene amplification of Tannerella forsythia was done byusing synthesized cDNA as template for PCR reaction.
Results: Tannerella forsythia was detected in 5 oral squamous cell carcinoma tissuesamples and was not found in any of the control tissue samples.
Conclusion: Tannerella forsythia could play a role in the pathogenesis of oral squamous cell carcinoma.

Keywords: *Tannerella Forsythia*; Oral Malignancy; Gene Amplification.

Introduction

Oral squamous cell carcinoma (OSCC), a subset of Head and neck squamous cell carcinoma (HNSCC), is the most common malignant oral neoplasm. OSCC accounts for 90% of all oral malignancies, and it has a poor 5-year survival rate that has not changed in decades. As risk factors, smoking, alcohol consumption and human papilloma virus (HPV) infection have been implicated. However, these risk factors alone have not been sufficient in explaining the incidence and aggressive behaviours of OSCC [1]. Thus, other factors, such as oral dysbiosis may play an important role in OSCC tumor development, progression and metastasis, yet this has not been well explored. Indeed, dysbiosis of the commensal oral microbiota and their subsequent invasion of the tooth supporting structures (e.g., the gingiva, periodontal ligament and bone) lead to the initiation and propagation of an inflammatory condition termed periodontitis or periodontal disease (PD) [2]. Individuals diagnosed as periodontitis were 3.7 times to develop OSCC, indicating that periodontitis was one of the risk factors of oral cancers. Our previous study found that periodontal pathogens, Porphyromonas gingivalis, and Treponema denticola, were prevalent in OSCC.

Treponema denticola, Porphyromonas gingivalis and *Tannerella forsythia* appears in late stages of oral biofilm development and comprises the bacterial "red complex" that is considered pathogenic in the

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etiology of periodontal disease. Other periodontopathogenic bacteria have been proposed for inclusion in the red complex [3]. Tannerella Forsythia is a Gram-negative, anaerobic bacterium. Described by Tanner and co-workers, it was referred to as Bacteroidesforsythus. Currently, it belongs to the genus Tanerella. The bacteria's breeding is not easy due to its demanding growth conditions [4]. Recently, mounting evidence suggests a causal relationship between *Tannerella forsythia* infection and the development of malignancies. Herein we report the presence of Tannerella forsythiain the tissue of OSCC based on PCR based amplification.

Materials and Methods

Patients and Tissues samples

There were 60 tissue samples collected in this study, 30 of which were from oral squamous cell carcinomas and 30 of which were from normal oral cavities. Prior to the initiation of the study. Institutional ethicalclearance was acquired. Tissue samples were collected after informed consent was obtained from the patients and control subjects. Both tumour and control tissues were washed twice with sterile 1X PBS (Phosphate Buffered Saline) before being transferred to a 2 mL microfuge tube containing Trizol reagent and kept at -20°C until further use.

Total RNA isolation from tissue

RNA was isolated using Trizol based RNA isolation procedures. From frozen tissue, 20 µm sections were made and collected in a frozen tube. Approximately 250 mg tissue material was dissolved in 5 ml Trizol(Thermo Fisher -India) and homogenised with amicropestle for one minute. After adding 1 ml chloroform and mixing for one minute, the suspension was centrifuged at 12, 000 rpm for 10 minutes. A second phenol/chloroform extraction was performed, followed by an isopropanol precipitation. The air-dried pellet was dissolved in 100 µl double distilled water and further purified with a RNeasy mini column (Qiagen GmbH, Hilden, Germany), according to the manufacturer's instructions.The isolated RNA (1 µg/lane) was electrophoresed for three hours at 50 V on a 1% agarose/formalin gel, and stained with ethidium bromide to assess the quality of the RNA.The concentration of isolated total RNAs was determined by measuring absorbance values from wavelengths at 230, 260 and 280 nm using the NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, USA). Purity of the total RNA was estimated by calculating the A260/A230 and A260/A280 ratios to evaluate the levels of protein and polysaccharide/phenolic compound contamination, respectively.

cDNA synthesis

First-strand of cDNA was synthesized with total RNAs (2.5 µL) by using cDNA Synthesis kit (Thermo Scientific RevertAid First Strand).

Amplification and identification of *Tannerella forsythia* specific gene

PCR was performed with Taq polymerase master mix (Ampliqon, Odense, Denmark). The following thermocycling parameters were used during for PCR analyses. These were an initial denaturation at 94 °C for 30 s, followed by 34 cycles of 94 °C denaturation for 15 s, 56 °C annealing for 35 s and 72 °C extension for 30 s, a final extension step at 72 °C for 10 min and an infinite hold at 10 °C. The resulting PCR products were then examined by electrophoresis in 1% agarose gel.

Copy number quantification

Gel snaps were processed with Image J, which turned the pixel concentrations of the bands into pixel intensity ratios. Because the stain binds more strongly to larger fragments than to smaller fragments, it was required to compensate for them by utilizing the stain. Correction values were calculated in order to quantify the amount of DNA present in each band. By staining the molecular weight markers, which had been separated on agarose and dyed with Orange G, the correction factor was established. These parameters were used to compare an experimental value to a forecasted value.

Results

Tissue samples from 20 male and 10 female volunteers with various periodontal diseases were used to investigate the prevalence of Tannerella forsythia. As a control, the same numbers of healthy tissues were employed. The average age of the patients was 55 for men and 47 for women. The template DNAused for the PCR experiment to detect Tannerella forsythiayielded a specified amplicon. Tannerella forsythia was detected in 5 (16.6%) among 30 subjects.For the positive control reactions, each reaction produced a single band of the predicted size, as shown in Figure 1. Tannerella forsythia was found to be positive in five oral squamous cell carcinoma tissue samples, indicating that the bacterium may have an association to oral squamous cell carcinoma.

Copy number quantification

By analyzing the band intensities with Image J software, we were able to quantify the copy numbers, as shown in Fig. 2. In all the 5 samples, the copy numbers were more than8 *10.

Discussion

Oral cavity is one of the well-studied microbiomes till date with a total of 392 taxa that have at least one reference genome and

Figure 1. PCR amplified product of *Tannerella forsythia* in OSCC patients cDNA sample. Lane L, molecular size marker (100-bp DNA ladder).

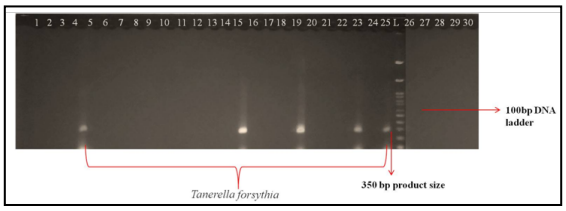
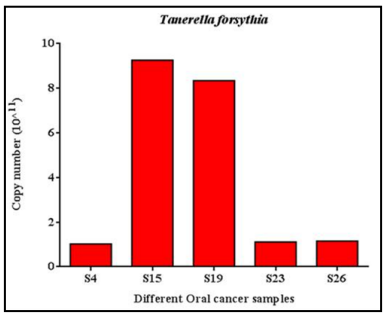


Figure 2. Copy numbers quantified based on Image J.



the total genomes across the oral cavity approaching 1500 [5]. Tannerella forsythia has been found to be associated with an increased risk of oral cancer and holdsnumber of signalling pathways responsible for cancer development. *Tannerella forsythia* yield several virulence factors and immune evasion factors, instigating inflammation and destruction of periodontal tissues [6-8]. The multifactorial aetiology of cancer formation should also be considered, and the role of bacteria should be seen as a key, but not the most important, aspect in this regard.In this current study, 5 patients were showed positive for the presence of Tannerellaforsythia in oral cancer tissue, and we summarized the associations between these bacteria and incidence and prognosis of oral cancer. Moreover, there is extensive evidence showing that Tannerella forsythia and Porphyromonas gingivalis are abundant in tumors and activate transduction pathways, such as anti-apoptotic pathway and nuclear factor-ϰB, leading to prognosis of cancer. Improving oral hygiene and treatment of periodontitis can significantly reduce the occurrence of oral cancer.

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