RNA SEQUENCING ANALISIS OF PERI-IMPLANT TISSUES: A SPLIT MOUTH STUDY

INTRODUCTION

Dental implants have become one of the most widely used tools in the rehabilitation of partially or totally edentulous patients. Despite the widespread use of dental implants, this type of therapy is not without complications. These can be divided into early complications, such as surgical trauma and intra-operative infections, and late complications, such as peri-implantitis (PI). PI affects both hard and soft peri-implant tissues with a prevalence rate of 15-25% in both academic and private practice settings. PI may initially manifest itself with milder symptoms such as tissue inflammation, spontaneous and probing bleeding and demineralisation of the peri-implant bone, but it can also progress if not promptly diagnosed or inadequately treated to the point of implant loss and massive destruction of the surrounding bone. Over the years, it has also been investigated whether there might be genetic factors that predispose to peri-implant disease, thus making a subject or an implant site more susceptible to the development of peri-implantitis due to a particular genetic pattern expressed by the tissues. This genetic predisposition would also provide an explanation for the development of the disease in those patients without other risk factors and would allow us to define better the genetic basis of this disease and potentially develop a more effective treatment than those that currently exist. The aim of our study is therefore to define whether there is a different gene expression profile in tissues with peri-implant disease compared to healthy peri-implant tissues.

MATERIALS AND METHODS

Our research project is a retrospective split mouth study examining the peri-implant tissues of patients who received implant-prosthetic rehabilitation with at least two implants, one of which is affected by peri-implant disease and one of which is healthy. The split mouth design of the study will allow to exclude all genetic variables normally present between different individuals, thus reducing the sample size and highlighting differences in gene expression between healthy and diseased tissue in the same patient. The samples will be analysed by a researcher other than the one who will be perform the specimen collection and will be identified with an alphanumerical code to ensure blindness. Patients with no risk factors for the development of peri-implant disease will be included for this retrospective study on the basis of the following inclusion criteria: systemically healthy patients, not assuming drugs or medication affecting bone metabolism, non-smokers, with good oral hygiene habits. These patients receive partial implant rehabilitation with at least two implants. These rehabilitations will be performed correctly, without obvious technical errors and without masticatory overload. To be included in the study, each patient shall have one implant affected by peri-implant disease according to the diagnostic criteria of the European Federation of Periodontology and one healthy implant. The gene expression within the peri-implant tissues will be assessed through RNA-Sequencing (RNAseq), which, based on recent Next-Generation Sequencing (NGS) technologies, analyses the transcriptome, i.e. the set of RNA molecules present in a cell and their quantity. This technique has been chosen, as it allows differential expression analysis, i.e. the identification of genes that show significant differences in their expression level

between two or more experimental conditions, for example between a sample of healthy tissue and a sample of diseased tissue from the same individual, as in our case. From each patient, two peri-implant tissue samples will be taken at the level of the keratinised mucosa approximately 5x1 mm in size, comprising both epithelium and peri-implant connective tissue, one from the diseased site and one from the healthy control site. The precise design of the extraction will be evaluated on a case-by-case basis to minimise possible complications and allow for the best possible healing. Once extracted, the samples will be fixed in an RNA-stabilising solution and stored at 4 degrees for 24 hours before analysis to avoid RNA degradation. RNA will be then extracted from the cells and subjected to the sequencing process using commercially available kits. Differentially expressed genes (DEGs) will be calculated using DESeq2.

RESULTS:

Genetic analyzes highlighted variations in gene expression between healthy samples and diseased samples of approximately 1100 genes in at least one patient. The genes that varied in all three patients were 13 but the genes whose variation was uniform and statistically significant were 3. These three genes are:

1)ENSG00000106483_SFRP4.The ENSG00000106483 gene encodes the SFRP4 protein, which is part of the family of soluble epidermal growth factor (EGF) receptor-binding proteins. Proteins of the SFRP (Secreted Frizzled-Related Proteins) family are involved in the regulation of embryonic development, cell growth and differentiation.

2)ENSG00000164694_FNDC1. The ENSG00000164694 gene encodes the FNDC1 (Fibronectin Type III Domain Containing 1) protein, also known as "fibronectin domain-containing protein 1". FNDC1 is a protein with a type III domain similar to that of fibronectin, which is involved in a variety of biological processes.

3)ENSG00000232679_LINC01705. The ENSG00000232679 gene, also known as LINC01705, encodes a long noncoding RNA (lncRNA).

None of these three genes had yet been associated with the development of peri-implant disease and their role seems to indicate alterations in bone metabolism during the development of the disease.

IMPORTANCE OF THE FINDINGS

Identifying the differences in gene expression between healthy and diseased tissue, regardless of the disease being studied, answers an important question: what are the molecular bases underlying the development of a disease? The discovery of this mechanism may open the door to understanding the development, behaviour and outcome of peri-implant disease, especially in patients with no other predisposing factors. In addition, with time and proper study, understanding the genetic basis of peri-implant disease could help developing a

more effective therapy for each individual patient. In fact, for many years now, the direction in medicine has been towards individualised genetic therapies, which are developed according to the specific characteristics of each individual and are therefore more effective. The development of personalised gene therapy based on the present approach and future research could significantly increase the success rate in treating a disease that affects millions of people around the world.