Porphyromonas gingivalis, periodontitis and oral cancer
The possible role of epigenetics in cancer development

Master thesis by Syver Andreas Vollan Rotberget and Alexander Salvador Guido

Supervisors: Inger Johanne Schytten Blix, Morten Enersen and Thomas M. Küntziger

Institute of Clinical Dentistry
Department of Periodontology
Institute of Oral Biology

FACULTY of DENTISTRY
UNIVERSITY OF OSLO

May 2018
Abstract

*Porphyromonas gingivalis* is an anaerobic bacterium heavily associated with periodontitis, a chronic inflammatory disease resulting in the irreversible destruction of the periodontium. With a vast array of virulence factors at its disposal *P. gingivalis* alters the surrounding environment according to its needs, promoting a dysbiotic microbiologic milieu that may also play a role in local carcinogenesis. There is currently an accumulating body of evidence regarding *P. gingivalis’* involvement in several types of orodigestive cancers, such as oral squamous cell carcinoma (OSCC), oesophageal cancer and pancreatic cancer. Recent research has also discovered that bacteria are able to directly intervene with the epigenetic landscape of infected host cells, in a manner appropriately nicknamed pathoepigenetics. In the following article we will discuss *P. gingivalis’* virulence factors and the bacterium’s associations with cancer development, with a main focus on epigenetics. Furthermore, we will discuss possible cancer mechanisms in this context, from the knowledge of similar bacteria-associated cancers. Starting out however, we will lay down the very basics of DNA-organization and epigenetic regulation and also give a brief summary of periodontitis.
# Table of content

Abstract .......................................................................................................................... 2
Table of content ............................................................................................................. 3
Preface ............................................................................................................................. 4
Introduction .................................................................................................................... 5
Deoxyribonucleic acid (DNA) ...................................................................................... 6
Epigenetics .................................................................................................................... 9
Periodontitis .................................................................................................................. 13
*Porphyromonas gingivalis* ......................................................................................... 18
Inflammation and cancer .............................................................................................. 24
*P. gingivalis* and cancer association: knowledge from other oral bacteria ............... 28
A new field of research, pathoepigenetics ................................................................. 30
*P. gingivalis* and possible cancer mechanisms ......................................................... 34
Conclusion .................................................................................................................... 36
Acknowledgements ....................................................................................................... 37
References .................................................................................................................... 38
In the course of the last year, as dental students we have been encouraged to not only focus on teeth but also divert our attention towards other parts of the mouth in order to diagnose cancerous developments early on; given that early diagnosis and eventual treatment of abnormal changes in the oral cavity correlates with a better prognosis.

We wanted to write about a topic related to periodontitis, and as we talked to our supervisors we were encouraged to look into this relatively new and interesting field of research regarding microbiology and cancer.

This topic also provided us with the opportunity to include our original interest in writing about periodontitis, and that was how we ended up with the current theme for our thesis.
Oral cancer accounts for about 2% of all estimated new cancer diagnoses worldwide (Ferlay et al., 2015), and about 90% of these are OSCC. In Norway the incidence of oral cancers, including areas such as the lip, tongue, salivary glands and pharynx, accounted for 2.3% of all new cancer cases diagnosed in 2016 (Cancer registry of Norway, 2016). The survival rates for patients diagnosed with OSCC varies depending on the location of the tumor and the time of diagnosis, but the mortality rate is high in general with about a 50-60% 5-year survival rate (Siegel et al., 2016). The poor prognosis may be due to the fact that most of the patients diagnosed with OSCC have tumors with an already considerable size, due to the rapid progression of the tumor, and/or possible nodal metastasis at the time of diagnosis (Massano et al. 2006; Warnakulasuriya, 2009). Treatment is also often complicated due to the position of the tumor in the oral cavity, hindering easy access to the tumor, and/or metastasis into adjacent or distant tissues. Smaller tumors that have not yet reached a stage of metastasis may be treated with surgery and/or radiotherapy alone, metastatic tumors however are treated with a combination of radiotherapy and chemotherapy (Algazi et al. 2016). While cancers in their earliest stages may be completely removed by means of surgery and/or radiotherapy with a high probability of long-time survival, the patients typically may be plagued with life-altering side effects from the above-mentioned therapy (Tolentino, 2011).

The involvement of bacterial infections and their possible supporting role in cancer development and progression is currently a hot topic. Finding out whether and how bacteria can influence certain cancer developments may eventually result in new therapeutic approaches by utilizing these newfound mechanisms. In addition, detection of the implicated bacterial species may in the future be used as a diagnostic marker and could be a possible risk factor for cancer development.
Our genetic material resides inside the nucleus in the molecules of deoxyribonucleic acid (DNA). DNA contains all the genetic information necessary to create every single protein in our bodies and is therefore an absolute vital part in order to ensure proper growth, survival and reproduction.

The genetic code is said to be universal, which means that all living organisms on earth use DNA as a method to store their genetical information (Brown, 2002). Bacteria, Archaea and Eukaryota, the three domains as proposed by Woese et al., 1990 all use DNA for this purpose. In microbiology we distinguish between prokaryotic and eukaryotic cells. Prokaryotic cells are defined as cells that besides the cell membrane do not contain any intracellular form of membranes that segregates the organelles. In eukaryotic cells however, the organelles are separated from each other and the cytoplasm by means of individual membranes, for example the nucleus (Stanier et al., 1962).

The DNA molecule is made up of the following components: a pentose sugar; deoxyribose, a phosphate group and four different nitrogenous bases. The four nitrogen-containing bases are: Cytosine, Thymine, Adenine and Guanine. Cytosine and Thymine are single-heterocyclic ring molecules called pyrimidines. The remaining two bases, Adenine and Guanine, are two-heterocyclic ring entities called purines. Combination of these make up a nucleotide which acts as the monomer unit in DNA, linked together in long chains. Distinct sequences of nucleotide bases define different amino acid sequences and thereby different proteins. The DNA-molecule is held together by strong phosphodiester bonds between the sugar molecules and the phosphate groups.

In 1953 Watson and Crick postulated their now famous design for DNA, the double helix (Watson et al., 1953). Two long strands of nucleotide polymers spiral to create the structure, as shown in figure 1. If we think of DNA as a ladder, the sides would consist of the sugar molecules and phosphate groups and the nitrogenous bases would represent the rungs. In the DNA molecule, the nitrogenous bases are organized in a locked pattern: Cytosine always pairs up with Guanine and Thymine always pairs up with Adenine. The binding that occurs in between the nitrogenous bases are weak hydrogen bonds. This complementary base pairing makes it so that even if one of the DNA strands is damaged or lost, the remaining thread will make repair possible by using that one as a template. In the same way, DNA synthesis is made possible by
unwinding the DNA and then using both threads as a template to synthesize two new DNA double helix molecules.

As depicted in figure 2, regulatory DNA regions called promoters will after binding of regulatory proteins initiate the transcription of the associated gene (Sainsbury et al., 2015). Just like promoters, other non-protein coding DNA regions such as Silencers, Enhancers and Insulators regulate gene transcription (Riethoven 2010). The processes of DNA transcription, synthesis and repair are tightly regulated in order to ensure that the integrity of the genetic material stays intact.

The human genome consists of approximately 3 billion base pairs and if fully stretched out, it would roughly equal a length of 2 meters! In eukaryotes DNA is safely tucked away within the
cell nucleus, protected by the nuclear envelope, shielding it from any hazardous influences. For the DNA to fit within the microscopic nucleus it needs to be compacted, DNA is therefore organized into several levels of compaction.

**Figure 2.** A simplified overview showing how the assembly and binding of transcription factors is needed in order for the RNA polymerase to be able to begin DNA transcription. Courtesy of OpenStax Anatomy and Physiology @2016

DNA is wrapped around a histone protein core consisting of several histone proteins creating an octamer and leading to a structure called a nucleosome (Richmond et al., 1984; Luger et al., 1997). The histone protein octamer consists of two copies of each of the four core histone proteins H2A, H2B, H3 and H4. In between the nucleosome complexes there are short segments of DNA called “linker DNA”, which are associated between nucleosomes to another histone protein called H1 linker protein. When using electron microscopy, nucleosomes looks like small “beads on a string” linked together by thin linker DNA bridges (Olins et al., 1974). The combination of DNA and histone proteins (with other associated proteins) is also called chromatin (Kornberg, 1974). Nucleosomes then form a helical solenoid structure, which is condensed further into chromatin loops that attach to a protein scaffold. A single chromatin loop contains almost 100,000 base pairs, or 100 kilobases. The result of this intense coiling is that the total size of the DNA is about 1/10 000th of what it would be completely stretched out.

Chromatin is arranged into tight electron-dense regions called heterochromatin, and more relaxed electron-light areas given the name euchromatin (Huisinga et al., 2006). Heterochromatin represents a rather gene-poor area where transcriptionally silent regions make up the main bulk. Euchromatin on the other hand consists of gene-rich and transcriptionally active regions. Why these two genetic landscapes have exactly these properties results partly from their epigenetic signatures, an important gene expression regulation mechanism.
Epigenetics

According to Robin Holliday the term epigenetic was first introduced by biologist and geneticist Conrad Waddington in the 1940’s as a way to reconcile the fields of developmental biology and genetics, which were two separate disciplines (Holliday, 2006). In that account, Waddington believed that embryology was not different from genetics and changed the greek word epigenesis to epigenetics to deepen the understanding of the processes involved in cell differentiation in early embryos (Waddington, 1939).

In epigenesis it was proposed that the early embryo is undifferentiated and development involves the execution of a complex developmental plan mediated by chemical reactions (Holliday, 2006). Nevertheless, epigenesis alone could not give answers to the questions such as how a stem cell could give rise to another undifferentiated stem cell and to a differentiated cell, or how a stem cell could develop into a variety of specialized cells with the ability to continually reproduce themselves through mitotic division and maintain their cellular and physiological phenotype traits. Another phenomenon that needed clarification was the inactivation of one of the X chromosomes in female mammals in the early stages of embryo development. If all cells in one organism have the same DNA and originate from a single undifferentiated stem cell, a mechanism must exist to allow this process to take place without alterations in the nucleotide sequence in DNA.

The term epigenetic derives from the Greek prefix epi which means “on top of”, or “around”, and it first emerged as a way to understand the mechanisms by which a fertilized egg could become a complex organism (Felsenfeld, 2014). Given that cell differentiation is characterized by the expression of certain genes and the inactivation of others, the concept of epigenetic regulation implies that switching a gene on and off is dependent on structural elements that directly mark the corresponding DNA sequence for its transcription or silencing. Such features include methyl groups that can be bound to nucleotides cytosine (and adenine) in mammalian DNA in a process called DNA methylation.

Epigenetic mechanisms include not only the superimposing of methyl groups on to the DNA, but also changes in chromatin structure such as histone modifications that allows the unfolding of the chromatin and the modification of DNA condensation (Figure 3). For example, histones undergo post translational modifications that modulate their interaction with DNA and other nuclear proteins: histone tails protruding from the nucleosome can be modified by the addition
of different chemical groups and this may result in the unwinding of the chromatin (Dupont et al., 2009). Once the chromatin is uncoiled, different DNA sequences can be accessible to regulatory or transcriptional proteins. These changes in histones represent a major epigenetic mechanism.

Figure 3. Figure shows epigenetic mechanisms and possible effects on health. Borrowed and adapted from National Institutes of Health. [Link](http://commonfund.nih.gov/epigenomics/figure).

Epigenetic changes around the DNA or histones are facilitated by the action of various enzymes that covalently bind or remove chemical groups to these molecules. The most common epigenetic change on DNA is the transfer of a methyl group to the cytosine base by a methyltransferase enzyme. Changes in histones include methylation, acetylation, phosphorylation and ubiquitination or the removal of these compounds from their N-terminal tails, though modifications can occur in other sections of these proteins (Biswas et al., 2017). At the same time these alterations are found to be heritable through both mitotic division in somatic cells and meiotic division in germ cells (Holliday, 2006).
It is important to point out that epigenetic modifications are subject to changes in response to changes in the environment of the cell. In other words, hormones, growth factors and other chemical elements that come in contact with the cell can influence the pattern of epigenetic labeling on chromatin. This may result in activation or silencing of genes and may or may not result in detrimental effects in the physiology of the cell, depending on the DNA sequences affected. Along the same lines, there has been an increasing interest in studying how epigenetic mechanisms can explain the pathogenesis of illnesses such as diabetes or cancer; and how our knowledge of these processes can help us find different approaches to treat them.

Cancer cells are defined as cells that have lost their control over the critical processes of cell death and replication. This loss of control causes the cells to rapidly replicate, eventually forming the solid masses that are tumors. It has been proposed that more than 300 genes and gene products are epigenetically modified in several human cancers, which have been linked to increased cellular dysplasia and atypia, invasive and metastatic malignancy and proliferative changes (Gronbaek et al. 2007; Gupta et al. 2006). A special set of genes called oncogenes and tumor suppressor genes are especially important when it comes to possible cancer development. Oncogenes are genes which are associated with cell growth, cell division and survival in general and thus have the potential to cause cancer when up-regulated (Vicente-Duenas et al. 2013). Tumor suppressor genes on the other hand are involved with processes that under normal circumstances help prevent unregulated cellular growth and division and promoting DNA repair (Lee et al. 2010). Some of the most well-known oncogenes and tumor suppressor genes include ERBB2, H-RAS, RB and TP53.

Over the course of a long period of time our cells have acquired a highly efficient DNA repair system that repairs spontaneous damage to our genetic material, but sometimes this system does not manage to correct the errors that occur. If the repair of DNA damage is insufficient it will most likely result in some aberration, such as point mutations, deletions, duplications and insertions among others (Lahtz et al., 2011). The accumulation of such DNA damage is known to play a role in the development of cancer (Jeggo et al., 2016). Deficiencies in one or several of these DNA repair pathways themselves are naturally associated with a higher level of DNA damage.

Nowadays, processes like X chromosome inactivation, gene silencing, histone modification, imprinting, DNA methylation, cell differentiation and pathogenesis are understood in the context of epigenetics. In light of new advances in the field of molecular biology, the term has undergone changes in an attempt to incorporate the broadened knowledge of the genomic
operating system. As proposed by Wu et al. 2001, the concept of epigenetics has evolved into the following definition: “the study of changes in gene function that are mitotically and/or meiotically heritable and that do not involve a change in the sequence of DNA” (Wu et al. 2001). Even though we still have a long way to go to completely understand the way the cell works, the study of epigenetic processes seems to have opened the door to a new way of understanding the functioning of the genomic machinery.
**Periodontitis**

The periodontium (peri = around, odontos = tooth) (Lang 3) constitutes the supporting and anchoring frame of the teeth. It undergoes certain changes with age and is, in addition, subject to morphologic changes related to functional changes in the oral environment (Lang 3). It consists of four types of tissues that support the teeth to both the maxillary and mandibular bone: Gingiva (G), periodontal ligament (PL), root cementum (RC) and alveolar bone (AP) (Figure 4). Periodontitis refers to an inflammatory condition affecting the periodontium, which, if left untreated, may result in the periodontium’s progressive and irreversible destruction, ultimately leading to tooth loss.

![Figure 4](image)

*Figure 4. Depiction of the main tissues that make up the periodontium. Adapted and borrowed from Clinical Periodontology and Implant Dentistry, 6th edition. Oxford: Wiley-Blackwell; 2015.*

The gingiva alone can be subject to inflammation; a condition known as gingivitis. There is a strong correlation between the occurrence of periodontitis and gingivitis, where the latter will most likely be present in the early, established and advanced state of uncontrolled periodontitis. However, not all gingivitis will necessarily develop into periodontitis. Gingivitis is reversible after a thorough cleaning and removal of supra gingival plaque.
The main triggering factor for the development of both, gingivitis and periodontitis, is the bacteria and their products in dental plaque. Dental plaque is a form of biofilm, which is defined as matrix-embedded microbial populations, adherent to each other and/or to surfaces or interfaces (Lang 171). This extracellular matrix is composed of exopolysaccharides, proteins and extracellular DNA (Kinane 2). Bacterial adhesion to the pellicle of the tooth surface initiates the dental plaque formation. Given that the oral cavity offers excellent conditions for bacterial growth, such as a stable temperature, moisture, and a constant supply of nutrients (Lang 192), formation of oral biofilms can be potentiated by for example, an unsatisfactory oral hygiene. Moreover, as dental plaque grows larger, it plays a prominent role in the pathogenesis of periodontitis.

It is estimated that in the mouth we can find between 700-900 bacterial species. Biofilms in the gingival margin and in the gingival sulcus can host a population of more than 500 different bacterial species or phylotypes (Aas et al., 2005; Lang 177). The majority of these bacteria are facultatively or obligately anaerobic (Kinane 2). As the biofilm grows, they start taking on three-dimensional structures characterized by the formation of channels; where water, nutrients, waste products and other chemical compounds such as DNA circulate. These compounds then become available to the distinct bacterial community for their use or disposal. The complex structural organization allows the biofilm to show tremendous metabolic and phenotypic variation (Nath et al. 2013).

Hence, at any point of time, our oral cavity is bathed with millions of bacteria and most of them are part of the normal or indigenous microflora in the mouth. Some commensal bacteria can become pathogenic if the microbial equilibrium is disturbed (opportunistic pathogens), as with periodontitis.

Socransky and his co-workers studied over 13000 plaque samples and found out that bacteria in the subgingival plaque live in complexes. They defined and divided these communities into five groups, based on their similarities. One of them, the so-called red complex, formed by *P. gingivalis*, *T. forsythia* and *T. denticola*, was found to have the strongest correlation with deep periodontal pockets and bleeding on probing (Socransky et al. 1998). It was also shown that bacteria in the orange complex had a close relation to the red complex (Socransky et al. 1998). (Figure 5)
Figure 5. Shows the different bacteria complexes found in the subgingival plaque by Socransky and his co-workers. Adapted and borrowed from Teles et al., 2013.

Bacteria in these microbial complexes can benefit from establishing synergetic relations with each other (symbiosis), helping each other in growth and survival. For example, the waste product of one bacterial species can be the energy source of another (Nath et al., 2013). Furthermore, bacteria are known to share genetic material; a phenomenon that can result in increased virulence and improved ability to tolerate environmental stress, antimicrobial agents and host defenses. On the other hand, antibiosis can also be found among bacterial species in the oral biofilm, caused by ecological changes or by a direct effect of another species. These two forms of interaction between the diverse microbial community in the biofilm play a crucial role in maintaining microbial homeostasis and defining bacterial demography at any point of time.

Under normal conditions, when the patient has a good plaque control and the amount of dental biofilm is small, the microorganisms in the periodontium have a dynamic and stable interaction with the host’s immune system, preserving the periodontal tissue homeostasis (Hajishengallis, 2015). Disruption of this stability may lead to dysbiosis, a term that describes a state in which the quantity or effect of pathogenic species within the microbial population is increased (Hajishengallis, 2015). In addition, an increase in the prevalence of pathologic bacteria will trigger a stronger immune response, which may contribute to further destruction of the periodontium.
It is therefore important to point out that the host’s immune response to pathologic bacteria also plays a crucial role in the progression of periodontitis. The human immune system is the entity in charge of protecting the body against foreign invaders or damaging stimuli. The term inflammation describes the mechanism the body sets in motion to fight off the source of tissue injury and ultimately reinstate normal tissue’s physiology. This process can be divided into an acute and a chronic phase, where we can find cells of both the innate and adaptive system interacting with each other as well as with cells from the surrounding injured tissue to finally re-establish tissue homeostasis.

The innate immune response is the first mechanism of defense that the body has to combat a wide range of pathogens (Hatice et al., 2015). Leukocytes, such as neutrophils and macrophages are the main cells of the innate response and are among the first agents to come to the affected area. Neutrophils have antimicrobial functions that help stop the propagation of pathogens, as well as collagenase, an enzyme that break down collagen in order to facilitate migration of immune cells. Macrophages, on the other hand, will also come to the site in order to clear the tissue from debris and apoptotic neutrophils by phagocytosis. Later in the course of an acute inflammatory process it is possible to see an increase in the migration of cells of the adaptive immune system such as lymphocytes and plasma cells towards the infected area. An acute inflammation phase will normally be resolved with the elimination of infectious agents and restoration of tissue’s functionality.

On the other hand, if removal of dental plaque by the host is not optimal or the immune response is defective or aggravated to the point where it is unable to stop microbial growth, bacterial infection may end up in a prolonged chronic inflammatory reaction. This process is characterized by fibrosis of the damaged tissue, which results in an impaired recovery of the tissue’s functionality. In addition, further destruction of the tissue such as loss of connective tissue as well as alveolar bone is due to the unresolved action of persistent pathogens and the ongoing activation of immune cells. Hence the removal of plaque is crucial to the improvement of the periodontal tissue.

In conclusion, periodontal disease results from the disruption of the otherwise, balanced microbial ecosystem in the oral cavity, which may result in the overgrowth of pathogenic species. This will trigger an inflammatory response in the periodontium, which can be understood as the mechanism that the body’s immune system has to fight the intrusion of microorganisms, reestablish periodontal homeostasis and repair damaged tissue. However, a prolonged inflammatory response may result in extensive damage of the periodontium,
especially if dental plaque is not removed. Other host-related elements such as genetics, age, socio-economic situation, environmental factors such as diet, smoking and systemic health have also been confirmed to contribute to the initiation and progression of periodontal disease.
Porphyromonas gingivalis

*Porphyromonas gingivalis* is a gram-negative, non-motile, obligate anaerobic, rod bacterium found in the oral cavity in humans. Due to its anaerobic nature it is mostly found in the gingival sulcus, or other niches within the mouth where the conditions favor its growth. The bacterium is asaccharolytic, which means that it is not able to metabolize carbohydrates and instead relies on the breakdown of amino acids in order to produce energy. Another necessity for the growth of this microbe is iron, which is acquired through the agglutination and lysis of red blood cells and subsequent absorption and storage of heme/hemin (Olczak et al., 2005). A second possible source of iron is through the gingival crevicular fluid (Mukherjee, 1985).

In the recent years *P. gingivalis* has been recognized as one of the main contributors to both the initiation and progression of periodontal disease (Hajishengallis et al., 2012). Alongside *Treponema denticola* and *Tannerella forsythia*, *P. gingivalis* is part of the “red complex” which is heavily associated with advanced periodontal lesions (Socransky et al., 1998). The bacterium was found to be present in 85,7% of subgingival plaque samples from patients with chronic periodontitis, compared to 23,1 % in healthy individuals in a study done by Yang and coworkers (Yang et al. 2004). Additionally, a strong positive correlation between *P. gingivalis* numbers and pocket depth has also been found (Kawada et al., 2004). It is important to remember that *P. gingivalis* is a part of the human microbiome, mainly detected in the oral microbiota. However, an increasing number of studies indicate its ability to invade into oral tissue cells and relocate to other parts of the body through the bloodstream and invading cells in other tissues (cardiovascular and brain tissue, joints and pancreas) (Inaba., et al 2010, Singhrao., et al 2015, Koziel., et al 2014, Michaud., 2013).

This opportunistic pathogen shows a remarkable ability to cause shifts in the local milieu, where the homeostatic commensal bacteria community becomes dysbiotic and promotes inflammation, even at fairly low colonization levels (Hajishengallis et al., 2011). In addition to being able to change both the amount and composition of the host microbiota, *P. gingivalis* is able to modulate and thus evade important immunologic pathways through cell invasion. Thereby it will be able to evade death and create an optimal environment for its growth (Zenobia et al., 2015; Maekawa et al., 2014; Hajishengallis et al., 2014). Indeed, the alteration of the host microbiota and the ensuing inflammation and tissue breakdown creates a more favorable environment for *P. gingivalis* by presenting a rich supply of nutrients (for example compounds
containing heme and degraded collagen peptides) and additional sites for colonization (Hajishengallis, 2014).

The bacterium harbors a number of virulence factors that allows invasion and utilization of host cells, but also evasion of the host immune system which prolongs the survival of the bacterium and increasing the likelihood of causing disease (Bostanci et al., 2012).

**Capsule:**

Some strains of *P. gingivalis* are encapsulated, surrounded by an outer layer of polysaccharides (also known as K-antigen) granting the bacterium structural stability. The capsule is important when it comes to recognition by and interaction with the surrounding environment (Aduse-Opoku et al., 2006). A total of 6 different capsular antigens have been found, which make up K1-K6 (Laine et al., 1997). A study done by Singh et al. (2011) showed that encapsulated strains in general elicited a reduced host response, reduced phagocytosis, enhanced bacterial survival, reduced dendritic cell maturation and showed a higher grade of virulence in comparison to non-capsulated strains (Singh et al., 2011).

**Lipopolysaccharide:**

Lipopolysaccharides (LPS) are a vital part of the outer bacterial membrane of gram-negative bacteria and consist of a polysaccharide moiety covalently bound to a lipid molecule (Usually referred to as Lipid A). The polysaccharide part of the molecule is made up of two components: (i) O-antigen, which is a long-chain polysaccharide built up of repeating units of one to seven sugars and (ii) an oligosaccharide core comprised of roughly ten monosaccharides (Hitchcock et al., 1986). The LPS molecule as a whole was long thought to be the main instigator of the host immune response, but it was discovered that Lipid A indeed was the structure responsible for stimulating the immune system and causing the subsequent immune response, thus being the bioactive region of LPS (Loppnow et al., 1990). In a study by Schromm et al. (2008) it was shown that the biological activity of LPS was dependent on the shape of the Lipid A portion (Schromm et al., 2000). As a virulence factor LPS is central when it comes to the recognition by the host immune system through activation of Toll-like receptor 2 and 4, which leads to the initiation of an intracellular signaling cascade and ultimately the release of several pro-inflammatory cytokines (for example tumor necrosis factor-α, interleukin-1β, interleukin-6 and interleukin-8) (Jain et al., 2000; Darveau et al., 2004). Aside from the immunologic importance of the molecule, LPS also gives the bacterium additional structural stability and provides a
selective permeable barrier that hinders the influx of hydrophobic molecules and toxic chemicals (For example detergents and antibiotics) (Nikaido, 2003).

**Fimbriae:**

Fimbriae are small proteinaceous surface appendages that are located in the outer membrane of bacterial cells. *P. gingivalis* expresses two distinct types of fimbriae on its surface, major (long) fimbriae and minor (short) fimbriae. The major fimbriae are made up of a protein subunit (FimA or Fimbrillin) encoded by the *fimA* gene, while the minor fimbriae consist of a subunit named Mfa protein and is encoded by the *mfa1* gene (Amano, 2010). The major fimbriae have been classified into six different genotypes (I-V and Ib) based on differences in the amino-acid sequences of the *fimA* gene coding for the subunit proteins (Fujiwara et al., 1993; Nakagawa et al., 2000; Nakagawa et al., 2002). Evaluating the pathogenicity of the different genotypes has shown that *fimA* genotypes Ib, II and IV correlated with stronger infectious symptoms and increased inflammatory destruction (Nakano et al., 2004; Amano et al., 2004).

The bacterium’s invasive properties are largely due to its major fimbriae (or rather the subunit FimA/fimbrillin) which may bind to β1-integrins on the surface of epithelial host cells, resulting in a rearrangement of the actin and tubulin cytoskeleton networks and subsequent internalization of the microbe (Yilmaz et al., 2002; Yilmaz et al., 2003). *P. gingivalis* can also invade macrophages, an important phagocytic white blood cell whose main role lies in the clearance of debris, foreign materials and pathogens. It does so by using its fimbriae to induce co-association between Toll-like receptor 2 (TLR2) and C-X-C chemokine receptor type 4 (CXCR4), undermining the host defense and exploiting the resulting lipid-rafts as an entry platform (Hajishengallis et al., 2006; Wang et al., 2008). The activation of CXCR4 grants the bacterium a way of infiltrating the host cell by inducing a high-affinity conformation of complement receptor 3 (CR3) that is used for internalization, in addition to this the intracellular crosstalk between CXCR4 and TLR2 results in a dampening of the production of nitrogen oxide which may contribute to reduced clearance in vitro and in vivo. (this happens in a manner which is dependent upon cAMP-dependent protein kinase A activation) (Hajishengallis et al., 2007; Hajishengallis et al., 2008; Hajishengallis et al., 2013)

*P. gingivalis* fimbriae also play a role in the formation of subgingival biofilms, as they are crucial components needed for co-aggregation between bacteria (Kuboniwa et al., 2009).

Some strains of *P. gingivalis* do not possess fimbriae and accordingly have a notably reduced ability to adhere to and invade host cells. (Lee et al., 1992; Sharma et al., 1993)
Proteases

A myriad of hydrolytic, proteolytic and lipolytic enzymes enables *P. gingivalis* to thrive in its environment by inducing both pro- and anti-inflammatory effects based upon the needs of the bacterium. About 85% of the total proteolytic activity of *P. gingivalis* stems from a special kind of cell surface cysteine proteinase called gingipains (Potempa et al., 1997). Gingipains are divided into two categories depending on their substrate specificity, arginine-specific and lysine-specific gingipains (Guo et al., 2010). The arginine-specific gingipains (also known as gingipain R) have trypsin-like activity and are able to degrade extracellular matrix components, including the integrin-fibronectin-binding, cytokines, immunoglobulin and complement factors (Curtis et al., 2001). There are two types of gingipain R, *RgpA* and *RgpB*. The lysine-specific gingipain (also known as gingipain K), only consist of a single version called *Kgp*. The gingipains are able to cleave some important T-cell receptors such as CD2, CD4 and CD8 and thus play an important role in the down-regulation of the cell-mediated immune response (Kitamura et al., 2002). They also have the ability to inactivate both pro-inflammatory (IL-12, IFN-γ) and anti-inflammatory (IL-4, IL-5) cytokines through proteolytic activity (Yun et al., 2001, Yun et al., 2002, Tam et al., 2009). The arginine-specific gingipains can cleave complement component 5 (C5) which results in the release of C5a and C5b. C5a plays a crucial role when it comes to the chemotaxis of polymorphonuclear cells, and the cleavage of C5 therefore contributes to the immune response (Wingrove et al., 1992). The lysine-specific gingipain on the other hand can cleave the C5a receptor on polymorphonuclear cells, which may result in a decrease in their recruitment (Jagels et al. 1996a, Jagels et al., 1996b).

Peptidylarginine deiminase

*Porphyromonas gingivalis* peptidylarginine deiminase (PPAD) is an enzyme unique to *P. gingivalis* capable of modifying the amino acid arginine into citrulline, another amino acid. This modification leads to the loss of a positive charge. It has been demonstrated that *P. gingivalis* strains that are PPAD-deficient are actually less virulent than their PPAD counterparts (Gully et al., 2014). PPAD is thought to play a key role in the initiation of rheumatoid arthritis (RA) by leading to citrullination of host proteins and creating citrullinated neoantigens. Presence of antibodies against citrullinated proteins is used as a marker for RA (Konig et al., 2015).

It is important to point out that endogenous peptidylarginine deiminases (PADs) are found in mammalian cells and take part in post-translational modifications of particular target proteins;
affecting in that manner, different cellular processes (Vossenaar et al. 2003). There are five types of PADs (PAD 1-4 and PAD 6), allocated in specific tissues and some of them have been associated with pathologies in their respective tissue cells, including cancer. (Mohanan et al. 2012). (Figure 6)

Figure 6. Possible working mechanisms of PADs in carcinogenesis. Adapted from Mohanan et al. 2012.

**Outer membrane vesicles**

Outer membrane vesicles (OMV) are small microvesicles that shed from the outer membrane of gram negative bacteria such as *P. gingivalis*. These lipophilic vesicles contain several bacterial products such as LPS, lipoproteins, nucleic acids (DNA and RNA) and peptidoglycans (Grenier et al., 1987, Haurat et al., 2011, Veith et al., 2014). Like miniature bombs the OMVs elicits a strong immune response in the host by directly delivering bacterial toxins and other components to the host cells (Mashburn-Warren et al., 2006). The OMVs have been shown to stimulate host macrophages, resulting in an increased secretion of several substances such as TNFα, IL-12p70, IL-6, IL-10, IFNβ and nitric oxide (Fleetwood et al., 2017). Several of these secreted substances are pro-inflammatory cytokines and may have a hand in establishing the
highly complex inflammatory niche that *P. gingivalis* thrives in. In addition, OMVs also activate macrophage inflammasome complexes through the increased levels of IL-1β (Cecil et al., 2017). In a study by Waller and coworkers it was shown that OMVs by *P. gingivalis* were able to promote monocyte unresponsiveness to live *P. gingivalis* through a selective abrogation of Tumor Necrosis Factor (TNF) (Waller et al., 2016). In this way, not only does the OMVs promote inflammation but they also seem to aid *P. gingivalis* in evading the immune system.

One prominent feature of *P. gingivalis* is the ability to disrupt innate apoptic pathways, and the bacterium has several means to achieve this. In primary gingival epithelial cells infected by *P. gingivalis*, chemically induced apoptosis is suppressed through the activation of the Jak1/Akt/Stat3 signaling pathways which in turn controls the intrinsic mitochondrial apoptosis pathways (Mao et al., 2007, Yilmaz et al., 2004). By inhibiting the activity of the proapoptotic protein Bad, the ratio between the antiapoptotic Bcl2 and the proapoptotic Bax is increased which blocks the release of the apoptosis effector cytochrome c (Yao et al., 2010). Another method of suppressing apoptosis is through the modulation of specific microRNAs. Followed by the infection of *P. gingivalis*, an upregulation of microRNA-203 results in the suppression of a negative regulator called SOCS3 which ultimately leads to suppression of apoptosis (Moffat et al., 2011). In addition to this, *P. gingivalis* secretes a nucleoside diphosphate kinase (NDK) that can hydrolyze extracellular ATP and thus impair the ATP-dependent apoptotic pathway via the purinergic receptor P2X7 (Yilmaz et al., 2008).
Inflammation and cancer

Inflammation is the protective mechanism by which our body fights off pathogens, responds to injury and eventually restores tissue's structure and function (Chai et al., 2015). This process comprises a network of immune cells and their molecular products that work together to create the physical conditions necessary to clear off foreign bodies and repair damaged tissues.

Under the acute phase of inflammation, the host's antigen presenting cells (APCs) located in the area of injury are activated by compounds that are associated either with pathogens or with host-related injury or cell damage (Abbas, 2009). Activation of these cells occurs via binding of antigens to surface receptors on APCs. This interaction leads to intracellular signaling cascades that ends up with the transcription and release of inflammatory mediators such as cytokines and chemokines and growth factors. This results in vasodilation which again facilitates the extravasation of leukocytes neutrophils and other cells and molecules out in the interstitial space. A successful acute phase, will end up in the clearance of pathogens or damaged cells and restoration of tissue homeostasis. This process is also achieved by the release of anti-inflammatory cytokine by immune cells. On the contrary, if the acute inflammatory response fails to restore tissue normal functionality, this process continues into its chronic phase, where complete healing of the tissue is not achieved and instead, there is a continuous activation of the immune system. As a consequence, and in its futile attempt to get rid of the cause of injury, the immune system continues to release chemical compounds that not only affect target cells or microorganisms but also healthy tissue. That is when an inflammatory response shifts from being a mechanism of defense to being a contributor to further damage.

Nowadays, there is evidence linking chronic inflammation to a number of conditions, such as autoimmune disorders, cardiovascular illnesses, chronic obstructive pulmonary diseases, periodontitis and more recently cancer (Shanmugam et al., 2013). The notion that inflammatory processes might be involved in the development of neoplastic tumors is first attributed to the German physician Rudolf Virchow, who in the nineteenth century described the infiltrate of leukocytes in tumor tissues (Balwik et al., 2001). At first it was believed that this infiltrate was a result of an anti-tumor immune response, but it has later been suggested that the presence of immune cells in neoplastic tissue can have both pro and anti-tumorigenic effects (Eliav et al., 2013).
After his observation, there has been numerous studies that corroborate the implication of some chronic inflammation in the development of cancer. It has also been suggested that acute inflammatory responses to tumor antigens may contribute to cancer regression, by clearing at an early stage, the proliferation of neoplastic cells; acting in that manner as tumor-suppressor (Grivennikov et al., 2010). On the contrary, chronic inflammation has been linked to predisposition and possible initiation of cancer, influencing the different stages of cancer development (Sethi et al., 2012).

Even though, a direct causative connection between chronic inflammation and carcinogenesis has not yet been confirmed, studies have brought to light crucial molecular and cellular reactions that take place in the complex crosstalk between chronic inflammatory processes and the tumor microenvironment (Elinav et al., 2013). Simultaneously, it has been proposed that inflammation may have a crucial role in cancer initiation through genomic destabilization; either by increasing DNA damage or by undermining DNA repair mechanisms (Elinav et al., 2013). In this model, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are the molecules proposed as possible initiator of genomic instability. ROS are produced by the mitochondrial respiratory chain and under normal conditions constitute an important signaling molecule in cell survival and proliferation, taking part in different signaling cascades such as MAPK and PI3kinase (Paul et al., 2012). Nevertheless, ROS and RNS have the ability to interact with intracellular proteins and DNA components to change their structures and consequently, alter the normal function of the molecules affected. Negative effects of increased amounts of ROS and RNS may include breaks in the DNA strand and single base mutations (Mantovani et al., 2008).

It has also been argued that once a tumor environment is established, cancerous cells can exploit different signaling pathways to their advantage. As mentioned earlier, inflammation cells release cytokines, that upon binding with specific receptors, can precipitate an intra-cellular reaction that may affect gene transcription and cell proliferation. Activation of Signal transducer and activator of transcription (STAT), in particular STAT3 by inflammatory interleukin IL-6 has been associated with increased expression of cyclin D1, cyclin D2 and cyclin B, which again promote cell division (Yu et al., 2009). In addition, activation of STAT3 also translates into an increase in the transcription of proto-oncogene MYC and anti-apoptotic genes (Elinav et al., 2013). Another pathway influenced by inflammation is the activation of transcription factor NF-kB, which in turn enhances pro-inflammatory responses, cell survival and proliferation (Ben-Neriah et al., 2011).
Another way inflammatory mediators may increase the probabilities for carcinogenesis is by down-regulating the transcription of mismatch repair proteins (MMR) or by reduction of its enzymatic activity by ROS (Schetter et al., 2010). In addition, it has been proposed that inflammation may cause epigenetic changes such as DNA hypermethylation, which may result in silencing of tumor-suppressor genes such as MMR and p53 (Hahn et al., 2008).

Metastasis of tumor cells can also be facilitated by means of increased permeability to neighboring tissues. This is accomplished partly by the process of vasodilation generated in the context of an inflammatory response, but also by increased transcription of matrix metalloproteinase (MMP) by immune cells. Furthermore, inflammation is tightly linked to angiogenesis, the formation of new blood vessels, which is crucial for the access to nutrients and disposal of cellular waste by tumor cells (Galdiero et al., 2013). Some of the above-mentioned mechanisms are depicted in figure 7.

![Figure 7. Potential cross-talk between inflammatory mediators during cancer initiation and progression. Borrowed and adapted from Chai et al. 2015.](image-url)
There is still room for studies that will reveal more about how inflammation can influence tumor initiation and progression. There is nowadays evidence that confirms the association between chronic inflammation and carcinogenesis and its possible mechanisms of action. It is clear that a tumor itself will elicit an inflammatory response, and the immune system will try to eliminate it. Nevertheless, it has been proven that an ineffective and prolonged inflammatory reaction can in fact tilt the scale in favor of carcinogenesis. It is calculated that around 20% of all cancer deaths are linked to chronic infection (Porta et al., 2009). In the context of bacterial-induced inflammation, it would be interesting to investigate the role bacteria may have in an inflammation-driven tumorigenesis and to know more about the crosstalk between bacteria, the immune system and the tumor environment.
There is an increasing amount of evidence that shows associations between periodontitis and several systemic diseases such as cardiovascular disease, diverse pregnancy outcomes, rheumatoid arthritis and diabetes (Hajishengallis et al., 2015, Preshaw et al., 2012, Hitchon et al., 2010). A possible link behind periodontitis and various types of cancer is also being investigated, with some attention focused towards *P. gingivalis* (Whitmore et al., 2014, Atanasova et al., 2014). Recent studies have shown a plausible association between *P. gingivalis* and several types of orodigestive cancers.

In the large European Prospective Investigation into Cancer cohort study, where the main goal was to evaluate the possible association between periodontal pathogen antibodies and pancreatic cancer risk, it was shown that individuals with high levels of *P. gingivalis* antibodies had a >2-fold increase in risk of pancreatic cancer (Michaud et al., 2013). Likewise, Fan and coworkers found a higher risk of pancreatic cancer in individuals who carried oral pathogens such as *P. gingivalis* and *Aggregatibacter actinomycetemcomitans* in a case-control study (Fan et al., 2016). The relationship between pancreatic cancer, the oral microbiome and periodontal disease is a plausible but complex one. Larger and more comprehensive studies are needed in order to definitely determine if and how the oral microbiome may contribute to pancreatic cancer risk (Bracci et al., 2017).

A recent study also demonstrated how *P. gingivalis* was detected in the cancerous tissue of esophageal squamous cell carcinomas (ESCC), while being undetected in normal healthy esophageal mucosa (Gao et al., 2017). In the same study the authors also detected lysine-specific gingipains, specific to *P. gingivalis*, as well as fragments of *P. gingivalis* 16S rDNA gene.

A study from 1998 found that human oral carcinomas harbored a significantly higher number of bacteria such as Porphyromonas, Fusobacterium, Veillonella, Actinomyces and more (Nagy et al., 1998). Likewise, a study by Katz and coworkers revealed that *P. gingivalis* was detected in higher levels in gingival squamous cell carcinomas than in healthy tissue (Katz et al., 2011). A highly important question to ask then is whether or not the heightened levels of bacteria may be a part of the carcinogenic process or simply a consequence of it.
The idea that bacterial infections, their bacterial products and the ensuing inflammation may influence carcinogenesis were generally neglected when first proposed, as opposed to well-defined risk factors such as genetics, tobacco use, age etc. After several years of continued research on the native bacterium *Helicobacter pylori* found in the gastric lining in humans, it became evident that there was a clear-cut connection between this bacterium and cancer development in the stomach. As such, in 1994 the World Health Organization (WHO) officially recognized the bacterium as a causative agent in cancer development. Following this, researchers all over the globe began to investigate other bacteria in order to discover new carcinogenic associations and their mechanisms.

One such example is the oral bacterium *Fusobacterium nucleatum* which is a commensal, gram negative anaerobe bacteria primarily found in the oral cavity and in the gut. It is established that *F. nucleatum* is involved in the development of periodontal diseases. Its implication in the onset and progression of other gastrointestinal illnesses such as inflammatory bowel disease and more recently to colorectal cancer (CRC) has also been documented (Yiping, 2015). In a similar way as with *P. gingivalis*, studies have shown significant presence of *F. nucleatum* in neoplastic and cancerous tissues as compared to healthy samples or tissues present in areas adjacent to tumours. (Castellarin et al., 2012)

Even though, the causative role of *F. nucleatum* in tumorigenesis has not yet been clearly demonstrated. There are studies that show the mechanism by which *F. nucleatum* can influence the tumour microenvironment around it. Studies on mice and APC (Adenomatous polyposis coli- a tumour-suppressor gene) mutated cells, showed that *F. nucleatum* had a pro inflammatory and tumorigenic effect. (Kostic et al., 2013) It does so with help of an adhesion molecule known as FadA. FadA is a surface adhesin that serves as a ligand for endothelial and epithelial cadherin, a transmembrane molecule responsible for holding cells together.

In the studies mentioned before, binding of FadA to cadherin molecules set off tumorigenic processes by initiating an intracellular signal response. This results in the translocation of β-catenin into the nucleus and subsequent activation of transcription factors for NF-κB, lymphoid enhancer factor and oncogenes Myc and Cyclin D1 (Leung et al., 2013). Simultaneously, FadA was found crucial for bacterial invasion of both endothelial and epithelial cells. In this case, invagination of *F. nucleatum* was more correlated with increased transcription of pro-inflammatory molecules, such as IL-8 and TNF-α. (Fardini et al., 2011)
A new field of research, pathoepigenetics

In the past few years several researchers have stumbled upon different species of bacteria that show a remarkable ability to directly intervene with the host’s epigenetic machinery. This enables the bacteria to tailor the surrounding environment to their own liking, subsequently granting them the upper hand against the host immune system and access to an abundance of nutrients. Some authors have begun referring to this phenomenon, the tampering and manipulation of the hosts DNA in an epigenetic fashion, as “Pathoepigenetics” (Cossart et al., 2014).

As discussed earlier H. pylori was the first bacterium to officially be recognized as a definitive risk factor for cancer. After coming in contact with the host cell it directly injects one of its main virulence factors, cytotoxin-associated gene A protein (CagA), by way of a Type-IV secretion system (T4SS). In the cytoplasm CagA can become phosphorylated by host cell kinases and thereafter interact with SHP2, which stimulates cell proliferation (Hatekayama, 2004). Another of its virulence factors, vacuolating cytotoxin A (VacA), has been shown to interfere with the proliferation of T-cells by inducing a G1/S cell cycle arrest (Gebert et al., 2003). H. pylori can induce histone modifications by several other means including modulation of the MAPK pathway. Dependent on the target cell type histones may either be dephosphorylated, deacetylated or phosphorylated following an H. pylori infection and these changes have been found to have an impact on both the cell cycle and transcription of oncogene c-Jun and heat shock gene hsp70 (Fehri et al., 2009, Pathak et al., 2006, Ding et al., 2010). Furthermore, studies have shown largely overlapping hypermethylation patterns between H. pylori-induced DNA hypermethylation in human gastric mucosa and in gastric cancer (Maekita et al., 2006).

A pore forming toxin from the bacterium Listeria monocytogenes called Listeriolysin O (LLO) induces dephosphorylation of histone H3 (H3deP) and deacetylation of histone H4 (H4deAc), which causes downregulation of a certain set of host genes (Hamon et al., 2007). In addition L. monocytogenes also secretes LntA factor which enters the host nucleus and interacts with the gene silencing complex protein BAHD1, resulting in a destabilization of the complex and subsequent transcription enhancement of the associated formerly repressed genes (Bierne et al., 2009, Lebreton et al., 2011).
Another example of a bacterial product that directly binds with DNA is ankyrin A (AnkA) secreted by *Anaplasma phagocytophilum*, a gram-negative bacterium which causes tick-borne fever in sheep and cattle. AnkA binds directly to DNA regions rich in AT-nucleotides and show special structural qualities such as the ability to uncoil chromatin under superhelical stress, for example regions in close proximity to the promoter of *CYBB* which encodes the gp91phox component of phagocyte oxidase (Park et al., 2004, Garcia-Garcia et al., 2009b). Binding of AnkA to the *CYBB* promoter results in the recruitment of histone deacetylase-1 (HDAC1), which in turn causes deacetylation of the region and ultimately silencing of the *CYBB*-pathway (Sinclair et al., 2014).

*Chlamydia trachomatis*, an obligate intracellular parasite and the cause of the sexually transmitted disease chlamydia, has a nuclear effector protein (NUE) which has been shown to enter the cell nucleus and associate with chromatin causing methylation of mammalian histones (Pennini et al., 2010). A clear-cut connection between NUE activity in the nucleus and changes in host gene expression is however lacking at the moment.

*Shigella flexneri*, a gram-negative bacterium and the main cause of shigellosis, can after infection of host cells secrete several bacterial effectors that translocate to the cell nucleus and interfere with important chromatin components. OspF modifies host MAPKs in an irreversible fashion by a reaction called eliminylation, which is the conversion of a phosphothreonine residue into a dehydrobutyrate residue that is no longer available for phosphorylation (Li et al., 2007). The phosphorylation of histone H3 at a set of NF-κB-regulated promoters is thus blocked and hinders the activation of certain proinflammatory genes (Arbibe et al., 2007). OspF and OspB can in unison bind the human retinoblastoma protein (Rb), which has the potential to recruit several chromatin-remodeling enzymes, resulting in a diminished host inflammatory response (Zurawski et al., 2009). Yet another secreted protein effector by the name IpaH9.8 has been shown to migrate to the host cell nucleus and promote degradation of an mRNA splicing factor called U2AF35 via ubiquitinylation, which in turn modulates the host immune response (Okuda et al., 2005). (See figure 8 for a summary of the different bacteria and their activities in the host nucleus.)
Figure 8. This illustration shows how several bacterial products, or “nucleomodulins”, enters the cell nucleus and interacts with the host machinery resulting in a change in gene expression. Borrowed and adapted from Bierne et al. 2009.

*P. gingivalis* has recently been shown to be involved in virus reactivation through epigenetic regulation mechanisms. Imai and coworkers established a connection between the bacterium and the reactivation of latent Epstein-Barr virus (EBV) in infected host cells, by means of chromatin modification induced by butyrate (Imai et al., 2012). Butyrate released from *P. gingivalis* results in the expression of the Epstein-Barr *BZLF1* gene product ZEBRA. The butyrate from *P. gingivalis* inhibits HDACs, resulting in an increased histone acetylation which
further down the line ultimately leads to a heightened transcription of the BZLF1 gene. In this manner, the bacterium may aid EBV reactivation in infected cells. (Figure 9)

![Figure 9](image.png)

**Figure 9. The synergetic relationship between *P. gingivalis* and the Epstein-Barr virus. Adapted from Imai et al. 2012.**

In another study LPS of *P. gingivalis* was shown to downregulate the expression of enzymes associated with epigenetic regulation in HaCaT keratinocytes. (Pereira et al., 2013). The enzymes that were downregulated were DNA methyltransferase 1(DNMT1), DNA methyltransferase 3a(DNMT3a) and histone demethylases Jumonji domain containing 3 (JMJD3). DNA hypermethylated promoter areas are associated with a decrease in gene expression in general, and so these findings may demonstrate how *P. gingivalis* alters the transcriptional machinery of the host in its own favor.
There is currently no evidence of a causal link behind *P. gingivalis* infection and cancer development. However, several studies have found associations between the bacterium and different cancer types. These associations are mainly based upon heightened levels of the bacterium, its products or components in precancerous and established cancerous lesions and the detection of bacterial antigens in the bloodstream of cancer patients.

Earlier in this work we reviewed *P. gingivalis*’ virulence factors and their effects on the surrounding environment, here we would like to discuss how these effects may contribute to the initiation and/or progression of carcinogenesis.

Given the recent studies that connect chronic inflammation and carcinogenesis, one could speculate that *P. gingivalis*, which is strongly associated with periodontitis, may play a role in carcinogenesis by means of initiating and maintaining a chronic state of inflammation. As mentioned earlier, it has been shown that inflammation may influence each of the stages of carcinogenesis. For example, it has been shown that *P. gingivalis* upregulates the expression of B7-H1 and B7-DC receptors in OSCC (Groeger et al., 2011). B7-H1 and B7-DC, otherwise known as programmed death ligand 1 and 2, are thought to contribute to immune suppression by reducing T-cell survival, cytokine production and proliferation (Greaves et al., 2013, Rozali et al., 2012). These receptors have been shown to be highly upregulated in cancer cells.

Another mechanism by which *P. gingivalis* may induce cancer is by inhibiting apoptosis, through different signaling pathways. Theoretically this may enable faulty cells, which have acquired unwanted oncogenic properties, to replicate unhindered. This may again result in accumulation of several genetic mutations and/or errors in the DNA repair process, causing the cell to further gain cancerous traits. Likewise, one could assume that the increased proliferation resulting from apoptosis inhibition in otherwise non-oncogenic cells may increase the risk of obtaining cancer-promoting genetic mutations.

*P. gingivalis* has been shown to enhance cell proliferation by means of accelerating S-phase progression of the infected cell. It does so by altering the proliferation pathways, upregulating certain cyclins and downregulating p53 (Kuboniwa et al., 2008) The p53 protein is a tumor suppressor that temporarily locks the cell in a cell-cycle arrest when it detects DNA-damage, thus stopping the cell from dividing before the damage has been repaired (Ozaki et al., 2013). In this way *P. gingivalis* infection may increase the risk of developing cancer by pushing a cell
through the cell cycle despite DNA damage. Another way *P. gingivalis* positively influence cell division is by cleavage and subsequent activation of the β-catenin signaling pathway via its gingipains (Zhou et al., 2015).

*P. gingivalis* peptidylarginine deiminases (PPADs) may have the potential to bring about similar effects on cellular activity as their mammalian homologs. This should be of special interest since it has been proven that citrullination of histone 3 (H3) and histone 1 (H1) by human PAD2 and PAD4 respectively, facilitates chromatin decondensation and subsequent gene transcription of IL-6, a pro-inflammatory cytokine. (McNee et al. 2017; Christophorou et al. 2014). There is also evidence that human PADs can affect other nucleosomal histones and that some cancer cells show upregulation of this enzyme (Mohanan et al. 2012). In that account, PPADs may favor carcinogeneis, depending on the genomic area accessible to transcription when PPADs unfold the chromatin. This could define *P. gingivalis'* epigenetic involvement in cancer.

Recently in a study by Inaba and coworkers it was demonstrated that *P. gingivalis* enhanced OSCC invasion through an increased expression and later activation of proMMP9 (Inaba et al., 2014). Matrix metalloproteinases (MMP) are a group of enzymes that are involved in the proteolytic degradation of extracellular matrix (Jablonska-Trypuc et al., 2016) The proMM9 overexpression effect of *P. gingivalis* in cancer cells is mediated by the proteinase-activated receptor 2 (PAR2). Following transcription of proMMP9 *P. gingivalis* gingipains proceed to convert them to their active form through proteolytic activity. Other signal pathways that also induce proMMP9 expression are ERK1/2-Ets11 and p38/HSP27, these were also shown to be activated during infection with *P. gingivalis*. In the context of cancer spreading, matrix metalloproteinases are crucial in allowing cancer cells to metastasize. Studies have shown that MMP2 and MMP9 in particular are tightly associated with cancer progression (Krüger et al., 2005).
Conclusion

The objective of this work was to find out more about the possible association between *P. gingivalis* and oral cancer. Considering bacterial species where a definite link with epigenetic alterations have been found, we tried to explore possible mechanisms that also could apply to *P. gingivalis*. In addition, we reviewed the molecular mechanisms that *P. gingivalis* may utilize to establish the conditions that favor either cancer initiation and/or progression. While there is still no identified epigenetic mechanism which *P. gingivalis* can use to directly interfere with the host cell nuclear components, it is clear that the presence of the bacterium in oral cancer cells has been shown to contribute to the process. It also remains to be found whether the increased levels of *P. gingivalis* in cancer tissue results from the tumor microenvironment offering an optimal milieu for growth, or vice versa.

There is a vast amount of literature that currently supports a plausible connection between *P. gingivalis* infection and cancer development. However, a causal link between the bacterium and carcinogenesis remains to be found. In order to discover this link, more extensive and elaborate studies are needed. Not only do we need more research on the bacterium and its effect on the tumor microenvironment in vitro, but also larger clinical studies that adjusts for other risk factors.

The discovery and understanding of such a mechanism could very well lead to further advancements in the prevention and treatment of bacteria-related cancers, by providing new therapeutic targets. At the very least, it would grant us an even better understanding of the complex interplay between microbe and host.
The authors would like to extend our heartfelt thanks to our supervisors Inger J. S. Blix, Morten Enersen and Thomas M. Küntziger for their steady support, critical feedback and for always being able to set aside time in their busy schedules to help us out with our work.
References


