TITLE: IDENTIFICATION OF A NOVEL LAMININ AND MATRIGEL LIGAND IN *PREVOTELLA*

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Abstract:

Prevotella spp. is a strict anaerobic bacteria associated with opportunistic infection in vaginal, mouth and gastrointestinal tract. During infection, adhesion to host tissue is essential for establishment and persistence of bacteria. Here we investigated interaction between strains of oral Prevotella species, *P. intermedia, P. melaninogenica and P. nigrescens* with laminina and matrigel. To evaluate binding, we allowed increasing concentrations of bacteria (5.10^7 and 10^7 UFC/mL) to interact with mouse laminin and matrigel immobilized in glass coverslips. We captured 10 random images of each coverslip [Field of vision (FV) = 0,049 mm^2] and quantified adherence. Initially, we identified adherence in *P. intermedia* and *P. nigrescens* species in laminin and matrigel. *P. intermedia* adherence was 491.154 UFC/FV and 191.231 UFC/FV, adhesion to negative control (1% BSA) was 6.61 UFC/FV. *P. nigrescens* adherence was 316,6 UFC/FV and 155,4 UFC/FV negative control was 98,1 UFC/FV. We did not detect any adherence of *P. melaninogenica.* To identify laminin ligands in *Prevotella*, outer membrane extracts were purified from *P. nigrescens* and ran through an NHS-activated sepharose affinity columns containing immobilized laminin. Eluted fractions containing potential ligands were analyzed by SDS-PAGE. Protein bands were excised from the gel and analyzed by mass spectrometry. We identified a 1481 amino acid putative adhesin protein (Accession number WP_040557291.1) which function as a laminin ligand in *P. nigrescens.* Blast search confirmed the presence of genes coding this protein in the genome of *P. nigrescens* as well as several other *P. nigrescens*. To our knowledge, this is the first time that binding activity is described for this putative adhesion. Next, we performed a transwell invasion assay for all the *Prevotella* strain. We saw that only *P. melaninogenica* was able to cross the matrigel. Considering this result, we decide to do a plasminogen activation assay. Ours results show that only *P. melaninogenica* is able to activate plasminogen and degrade matrigel with plasminogen. In the future, we hope discovery how *P. melaninogenica* is able to activate plasminogen and degrade matrigel, using kits with avidin/biotyn and zimografy analyses using matrigel and proteins of outer membrane. Understanding molecular mechanisms involved in adhesion may help us design new strategies to prevent periodontitis and biofilm formation in gingival sulcus.

Keywords: Adhesion, Laminin, Protein

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