

**Title:** Oral pathogens regulate epithelial cell behavior through the adherens junction-associated RNAi machinery

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**Introduction:** Increasing evidence portrays key roles of host-microbial interactions in the progression of epithelial diseases, including tumorigenesis. A pathogen that has been linked with tumor progression is oral microbe *Fusobacterium nucleatum* (*F. nucleatum*). However, the mechanisms by which *F. nucleatum* may influence epithelial cell behavior are not fully known. Notably, *F. nucleatum* has been associated with loss of epithelial integrity. The adherens junction (AJ) is an essential, E-cadherin-based, cell-cell adhesion complex that is key for epithelial tissue integrity but has also been implicated in regulation of cell behavior. Further, we have previously shown that epithelial AJs through the E-cadherin-p120 catenin partner PLEKHA7, recruit and regulate core components of the RNA interference (RNAi) machinery, such as DROSHA and AGO2, to suppress oncogene expression and pro-tumorigenic cell transformation. Thus, we sought to interrogate the AJ-associated RNAi machinery as a potential mechanism mediating the effects of *F. nucleatum* in epithelial integrity and pro-tumorigenic transformation.

**Methods:** We used a well-differentiated colon epithelial cell line model (Caco2), and assessed effects of *F. nucleatum* on AJs and other pro-tumorigenic markers. Caco2 cells were exposed to either heat-inactivated bacteria, or to bacterial supernatant from *F. nucleatum* spp. *nucleatum*, *Streptococcus salivarius* and *Escherichia coli* spp. *Nissile*. Cells were fixed after 8, 16, and 24 hours of incubation, and examined by immunofluorescence and confocal microscopy for changes in the junctional localization of E-cadherin, p120 catenin, PLEKHA7, AGO2, and DROSHA.

**Results:** Results showed that localization of PLEKHA7, AGO2, and DROSHA was disrupted by *F. nucleatum*, but not by *Streptococcus salivarius* or *Escherichia coli*. Also, these changes in localization were accompanied by upregulation of oncogenes such as JUN and SNAIL, as shown by western blot of cells treated with *F. nucleatum* supernatant for 24 hours. Oncogenes JUN and SNAIL were previously found to be suppressed by the junctional RNAi. This data supports that *F. nucleatum* may be promoting pro-tumorigenic transformation through disruption of the AJ-associated RNAi machinery leading to increased expression of oncogenes, and this can help deepen our understanding of the mechanisms mediating host-pathogen interactions in epithelial homeostasis and disease.