Streptococcus mutans antimicrobial genes mitigate oral cancer cell properties

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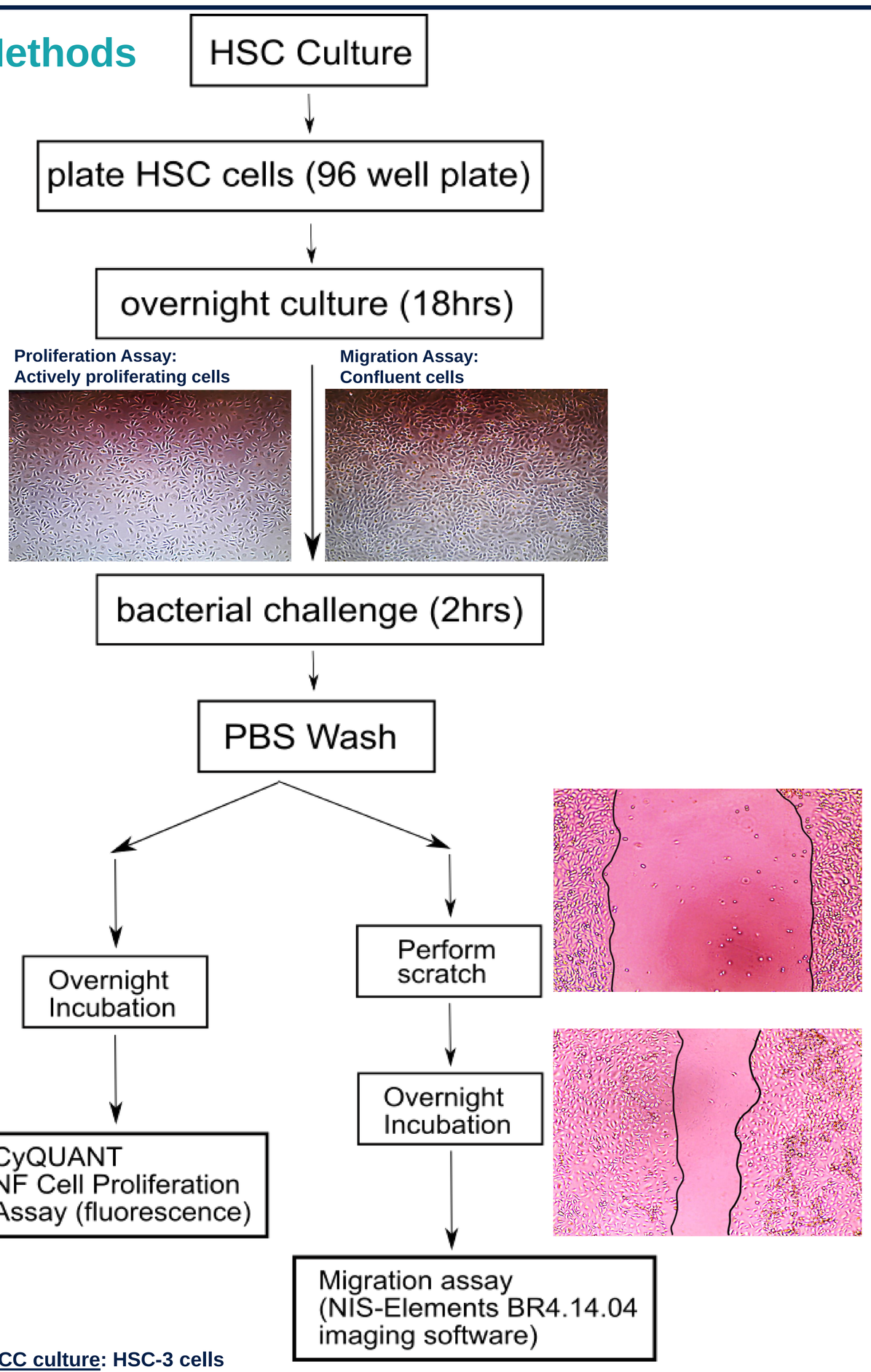
Introduction

- **Oral Squamous cell carcinoma(OSCC) is the 11th most common cancer worldwide with 300,000 new cases/year and a five-year survival rate of approximately 60% [1].**
- **Recent advancements in microbiome analysis have related dysbiosis of oral microbiota to OSCC diseased state [2, 3, 4].**
- **Oral commensal bacteria have been found to suppress OSCC activity while oral pathogenic bacteria promote OSCC cell progression *in vitro.***
- **Caries results from oral dysbiosis of overpopulation of *Streptococcus mutans* (SM), a common cariogenic species that can inhabit oral commensals.**
- **Poor oral health and hygiene habits have long been related to increased risks of OSCC and poor disease prognosis [5].**
- **NN2 and PNS1 are novel SM antimicrobial peptide genes, which were identified through full-genome sequencing of SM isolated from children with early childhood caries(ECC) and had following properties:**
  - ✧ **Assist SM to suppress oral commensal bacterial growth → dysbiosis of over-growth of SM and reduction of oral commensal bacteria → leads to caries development.**
  - ✧ **Are more prevalent in children with ECC than in caries-free children**

Aim

- **Investigate the role of antimicrobial peptide genes of SM in OSCC pathogenesis, specific to cell proliferation and migration.**

Methods



OSCC culture: HSC-3 cells
SM strains/ groups: NN2 WT, NN2 KO, and No bacteria control.
PNS1 WT, PNS1 KO, and No bacteria control.
Bacteria treatment concentration/time: 10, 50, 100 (MOI)/2hrs
Outcome measure:

- **Proliferation assays: fluorescence dye which binds to viable DNA, representing remaining oral cancer cells in culture. [6]**
- **Migration assays: area of scratch after overnight/18hr incubation compared to area of control scratch, measured from culture images. [7,8]**

Hypothesis

We hypothesize that presence of NN2 and PNS1 genes will promote OSCC cell proliferation and migration.

Results

Impacts of NN2 Gene on OSCC Proliferation and Migration

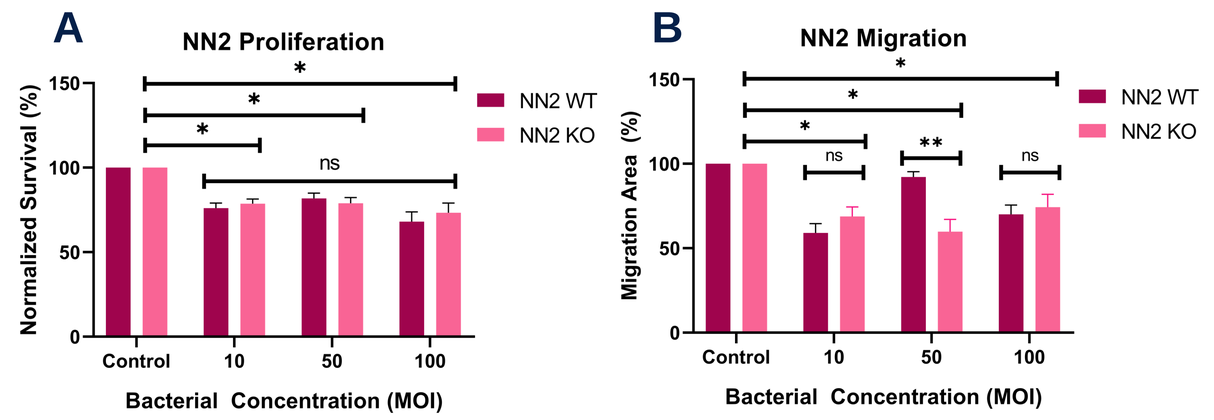


Figure 1.

A) The proliferation of OSCC cell treated by bacteria were slightly but significantly lower than non-infection control. However, there were no statistical significant differences on proliferation between NN2 WT and KO strains.

B) All OSCC cells treated with bacteria exhibited slight but significant inhibition of migration compared to non-infection control. We observed significantly more inhibition of OSCC cell migration by NN2 KO at 50 MOI(NN2 KO) than NN2 WT.

(Proliferation and migration of cells were measured via fluorescence of viable DNA from OSCC cells in the culture. Results were normalized against non-bacteria treatment control.)

Impacts of PNS1 Gene Proliferation and Migration

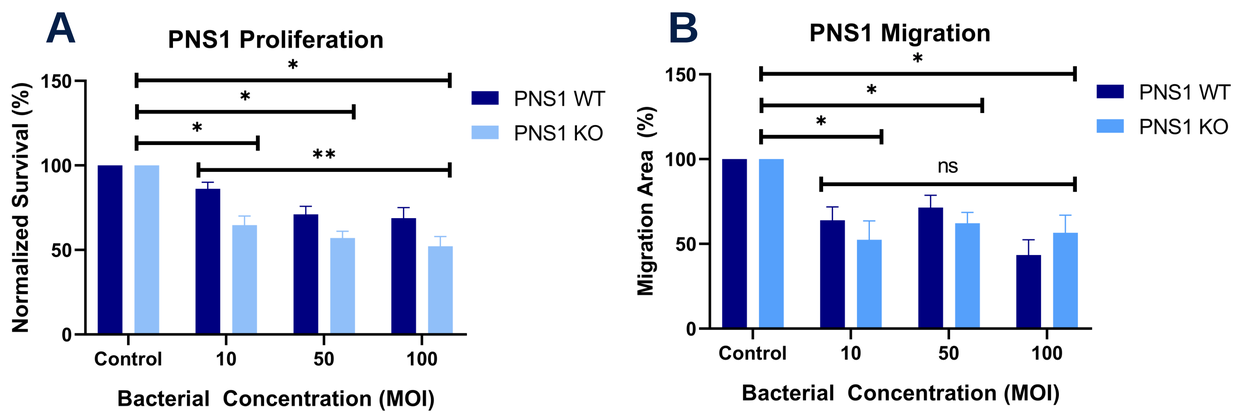


Figure 2.

A) The proliferation of OSCC cell treated by bacteria were slightly but significantly lower than non-infection control. We observed significantly more inhibition in PNS1 KO across all 3 bacterial concentrations than PNS1 WT.

B) All OSCC cell treated with bacteria exhibited slight but significant inhibition of migration compared to non-infection control. We observed no statistically significant differences between PNS1 WT and PNS1 KO strains in the migration of OSCC cells.

Conclusion

- **SM with the virulence gene NN2 and PNS1 exhibits minor inhibition (<30%) on OSCC cell in both proliferation and migration.**
- **However, when PNS1 or NN2 gene were knocked out, the KO strains exhibited significantly higher inhibition on OSCC cell proliferation and migration, respectively, indicating that these genes may help to mitigate the OSCC inhibition in different mechanisms.**
- **SM virulence factor genes NN2 and PNS1 are likely to be involved in OSCC pathogenesis through different molecular mechanisms.**

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