Tetraspanin 6 (Tspan6) regulates the attachment of *F. nucleatum* subspecies to colorectal cancer (CRC) cells

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1. Introduction

Several studies have shown an association of certain bacteria with CRC[1]. Our analysis of metagenomics data from 57 CRC tumour samples and their normal paired controls found a significantly higher abundance of certain bacteria in CRC tumours compared to controls**(figure 1).** These bacteria include the oral anaerobe *Fusobacterium nucleatum* **(figure 2)** which has recently been linked to CRC [2]. Several tetraspanin proteins are known to regulate the attachment of various bacteria to epithelial cells [3]. Tspan6 has recently been shown to have a role in the early stages of colorectal cancer (CRC) and its expression on CRC tumours was associated with increased patient survival [4]. This study aimed to determine whether Tspan6 expression had an impact on how three subspecies of *F. nucleatum*: ssp. *animalis* (FNA), ssp. *nucleatum* (FNN23) and ssp. *polymorphum* (FNP) attach to and invades epithelial cells in CRC.

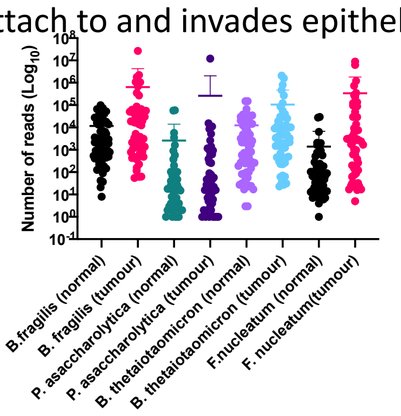

Figure 1: Comparison of the number of reads of certain bacteria in tumours vs controls from CRC samples.

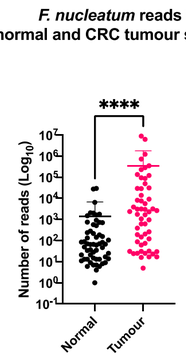

Figure 2. Number *of F. nucleatum* reads in normal vs tumours: A Wilcoxon matched paired signed ranks test was performed ****=p<0.0001) n=57

2. Experimental Protocol

✓ Invasion and adhesion assays were performed using Tspan6 positive and Tspan6 negative Caco-2cells, a well-established cellular model of CRC**. Figure 3** shows confirmation of Tspan (+) cells through western blotting.

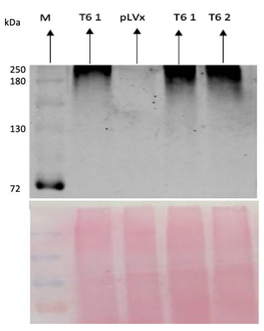

Figure 3. Western blots of T6 expression and ponceau image. Bands showing a signal in wells loaded with T6 lysates. No bands are present on the control pLVx wells.

Anaerobic bacteria culturing in the Don Whitley anaerobic chamber.

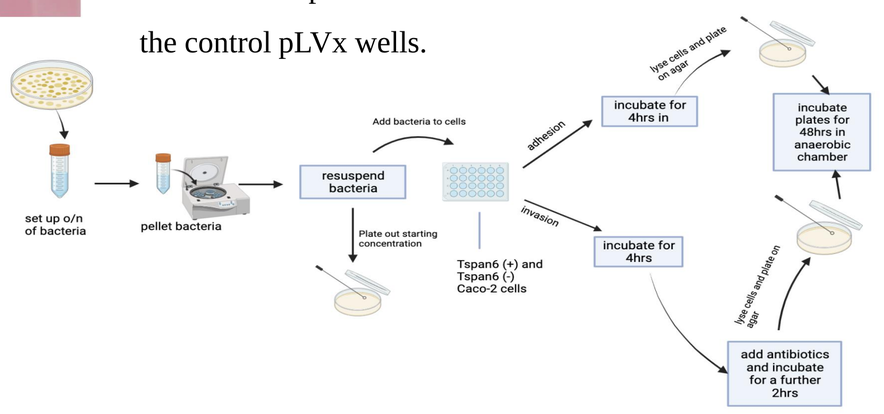

Figure 4. Schematic illustrating the experimental protocol for adhesion and invasion assays.

3. Results

3.1. Tspan6 expression reduces the attachment of FNP and FNA to CRC cells but increases the attachment of FNN23 to Caco-2 cells.

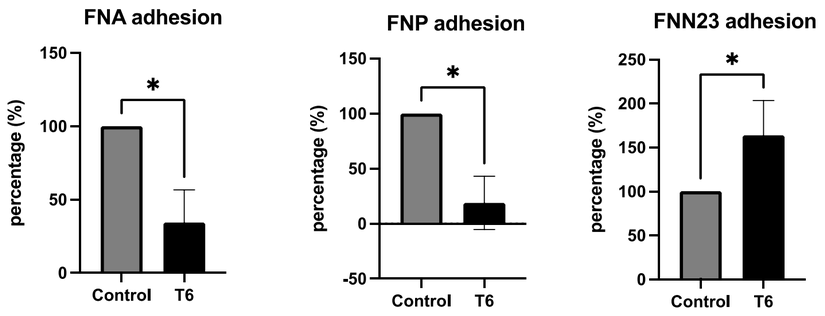

Figure 5. Adhesion of FNP (left) FNA (middle) and FNN23(right) to Tspan6 expressing cells compared to controls: Graph shows normalized to control and the mean and S.D of adhesion of T6 to Caco-2 cells. An unpaired two-tailed t-test was performed (*=p <0.05) n=3

3.2. Adhesion mutant 1 in FNN23 is important for the invasion of FNN23 into Tspan6 expressing cells.

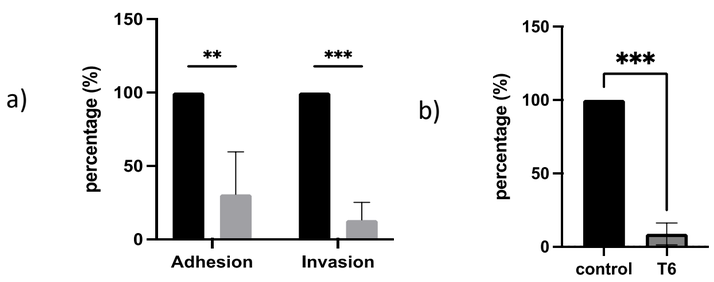

Figure 6. Decreased adhesion and invasion of epithelial cells by the adhesin mutant 1 compared to the wild-type strain(a). Tspan6 expression is important for the observed reduction in invasion by the mutant 1 strain **(figure b).**An unpaired two-tailed t-test was performed for the invasion of Tspan6 cells compared to controls. (*=p <0.05;**=p<0.001; ***=p<0.001) n=3

3.3.The adhesion mutant 2 in FNN23 is important for the adhesion of FNN23 to Tspan6 expressing cells.

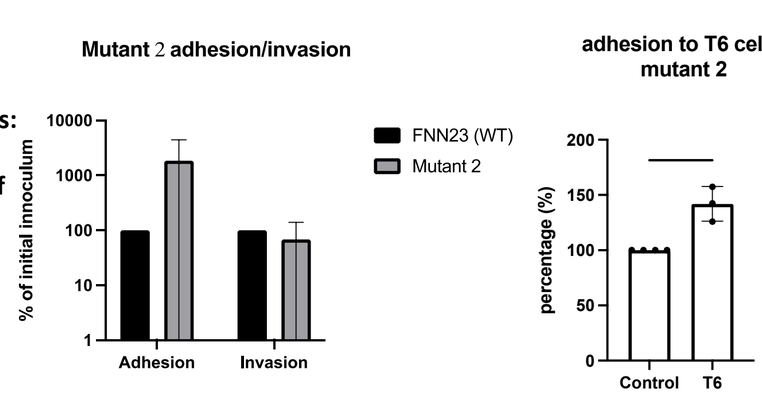

Figure 7. Adhesion and invasion by adhesin mutant 2 compared to the wild-type strain. Variation of the data for the mutant strain was observed compared to the WT. However, the mutant shows increased adhesion to Tspan6 cells (b). Unpaired two-tailed t-test was performed for adhesion to T6 compared to controls(b) (*=p <0.05) n=3

3.4. Bacteria growth phase impacts the adhesion of FNN23 and the mutant 1 adhesin mutant to cancer cells.

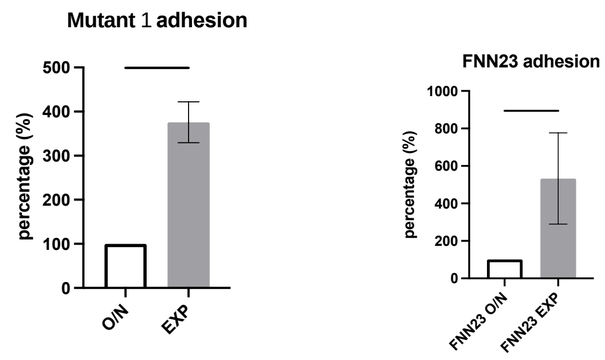

Figure 8. Comparison of adhesion (left) and invasion (right) of Tspan6 cells by WT vs mutant 1 strain. There is an increase in adhesion for cells at the exponential phase compared to overnight cultures (O/N) for both strains tested. An unpaired two-tailed t-test was performed (*=p <0.05;**=p<0.001; ***=p<0.001) n=3

5. Conclusions

- ✓ *F. nucleatum* subspecies differentially attach to CRC tissues.
- ✓ Tspan6 expression impacts the attachment of *F.nucleatum* subspecies to colorectal cancer cells.
- ✓ The adhesin mutant 1 is important for the adhesion of FNN23 to Tspan6 expressing Caco-2 cells.
- ✓ The adhesin mutant 2 is important for invasion of FNN23 to Tspan6 expressing Caco-2 cells.
- ✓ The bacterial growth phase has a key implication in the adhesion of bacteria to Caco-2 cells.

6. Future work

- ✓ Perform adhesion assays with *F. nucleatum* subspecies at the exponential phase to see if it impacts the rate of adhesion and invasion.
- ✓ Determine the expression profile of bacteria at the exponential phase compared to the stationary phase to determine the expression of adhesins at the different growth phases.
- ✓ Analysis of metagenomic data from 57 CRC samples and their normal paired controls as well as the RNA – sequencing data from these samples.

Collaborators

Dr Daniel Slade, Professor Andrew Beggs, Professor Steven Van Laere

7. References

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