Characterizing the Interactions of *Fusobacterium nucleatum* with Cancer and Immune Cells

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Abstract

The tumor microenvironment (TME) is composed of a complex collection of cells including not only malignant cells but also non-transformed cells such as fibroblasts, immune cells, and endothelial cells. In recent years, increasing evidence has demonstrated that the tumor-associated microbiota is a fundamental element of the TME, that can modulate the biological function of other eukaryotic elements within the tumor tissue. Microbiome analysis has shown that *Fusobacterium nucleatum* is highly enriched in colorectal cancer (CRC) tumor environments. Historically, *F. nucleatum* is present in the oral cavity of healthy individuals and is associated with periodontal diseases. High loads of *F. nucleatum* in CRC tumors has been associated with poorer patient prognosis, recurrence, and metastatic diseases, but little is known about the properties of *F. nucleatum* that lead to these correlations. In this investigation, we study the interactions of *F. nucleatum* with cancer epithelial cells and neutrophils through confocal imaging. We aim to compare the effects of an *F. nucleatum* strain isolated from the oral cavity of a non-cancer individual (oral strain) and an *F. nucleatum* strain isolated from a CRC tumor (CRC strain). We analyzed that the oral strain of *F. nucleatum* can also interact with epithelial cancer cells and neutrophils similarly to the CRC strain of *F. nucleatum*, suggesting that *F. nucleatum* species members have conserved properties in modulating cancer development.

Background

- *Fusobacterium nucleatum* is primarily found in the oral cavity of healthy individuals but in colorectal cancer (CRC) it is highly enriched in the tumor microenvironment.
- High loads of F. nucleatum is associated with poorer patient prognosis, recurrence, and metastatic disease.
- Genomic analyses has shown that there are genomic difference between the oral strain and the CRC strain of F. *nucleatum.*
- Research has focused on the associations between *F. nucleatum* and CRC but how does *F. nucleatum* give rise to these associations and are these properties conserved between the CRC strain and in its oral cavity counterpart?

Method

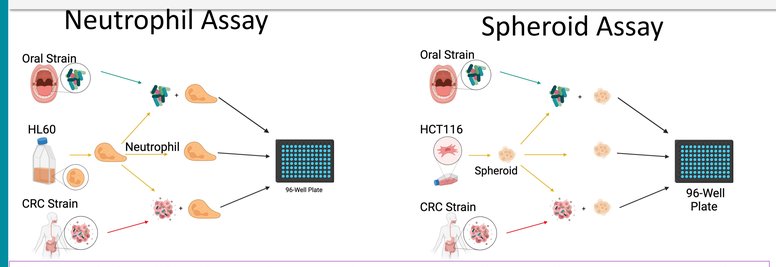

Fig.1-Description of Experimental Procedure: In both the spheroid assay (right) and the neutrophil assay (left) each *F. nucleatum* strain is cultured separately in blood agar plates. At the same time, tissue culture is done with cancer epithelial cells (HCT116) and neutrophils (HL60). Once the epithelial cells are confluent, they are trypsinized and seeded in a 96-well plate for the spheroid assay. The spheroids are seeded in three conditions: on their own (control), with the oral cavity strain, or with the CRC strain. The same procedure is followed for the neutrophils (right) except neutrophils are seeded in place of spheroids.

Results

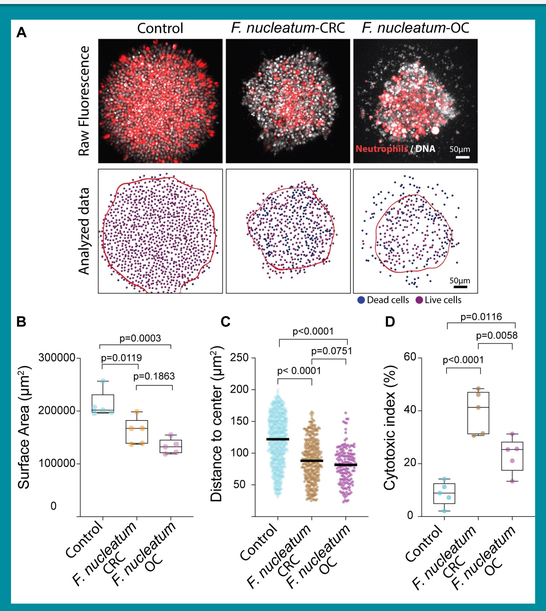

Fig. 2- Spatial Distribution and Cytotoxic Index of Neutrophils: A. Raw fluorescence (top) of the cytoplasm of neutrophils within each condition. When the data is analyzed (bottom), we see the distribution of neutrophils throughout the assay and the number of neutrophils alive (purple) or dead (blue). **B.** Box and whisker plot of the surface area covered by neutrophils in each condition along with the significance (p-value) of these results when one condition is compared to another. **C.** A plot of the distribution of individual neutrophils in the assay from the center of the well in each condition. Additionally, the significance of each conditions results is compared between groups through a p-value. **D.** Box and whisker plots characterize the overall cytotoxic index of the neutrophils in each condition and when conditions are compared, significance is demonstrated with a p-value.

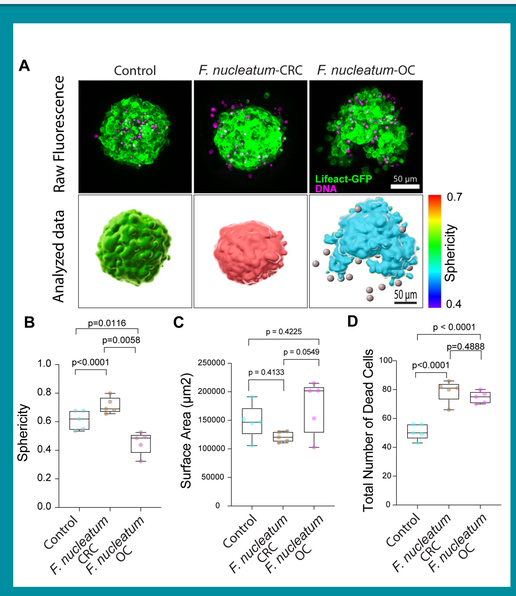

Fig. 3 – Interaction of CRC Epithelial Cells with *Fusobacterium nucleatum*: A. Raw fluorescence (top) of CRC epithelial cells (HCT116) expressing Lifeact-GFP (green) along with their dead counterparts (red) stained with PI. Analyzed data (bottom) of the fluorescence portrays the surface area of the spheroid as well as its level of sphericity within each condition. **B.** A box and whisker plot analyzing the level of sphericity between spheroids in each condition and identifying the significant difference of this observation between groups using p-values. **C.** A box and whisker plot shows the surface area of spheroids in each condition and if there is a significant difference between conditions. **D.** A box and whisker plot displaying the total number of dead CRC epithelial cells in each condition as well as the p-values relating the significant difference of dead cells between conditions.

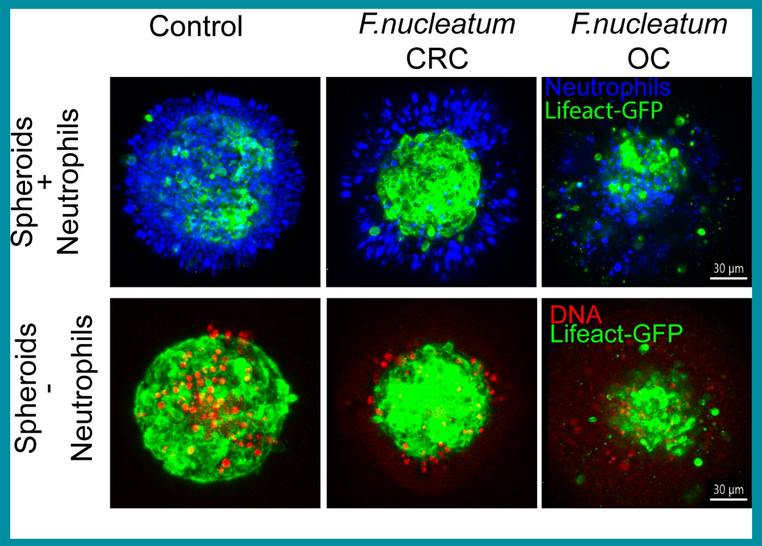

Fig. 4 – Confocal Imaging of Spheroids with and without Neutrophils:
(Top) Confocal imaging of spheroids in each condition, and visual observation of how neutrophils interact with them and *Fusobacterium nucleatum* in this environment. (Bottom) Confocal imaging of spheroids seeded in each condition without neutrophils to observe their interactions with *Fusobacterium nucleatum* and evaluate the visual difference in the number of dead cells (red) between conditions.

Conclusion

Through confocal imaging, we were able to compare the oral cavity strain of *Fusobacterium nucleatum* to the colorectal cancer strain. We found that both strains can interact with cancer epithelial cells as well as neutrophils in a similar manner, suggesting that members of the *F. nucleatum* species have conserved properties in modulating cancer development.

Future Directions

We plan to investigate the properties of these two strains further by repeating these assays as well as formulating new assays.

Acknowledgements

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