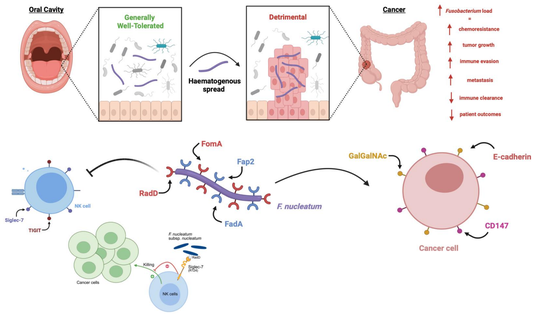
Immunodominant Proteins of Fusobacterium nucleatum: Comparative Analyses in Colorectal Cancer Patients, Healthy Individuals and Vaccinated Mice Models

***Petra Jozsa*[1,2]**, Nicholas Viegas[1,3], Cody Despins[1], Robert Holt[1,2,4]

[1]Canada's Michael Smith Genome Sciences Centre; BC Cancer; 675 W 10th Ave, Vancouver, BC, V5Z 1L3; Canada, [2]Department of Molecular Biology and Biochemistry; Simon Fraser University; SSB8166 – 8888 University Drive, Burnaby, BC, V5A 1S6; Canada,
[3]Department of Medicine; University of British Columbia; C201 – 4500 Oak Street, Vancouver, BC, V6H 3N1; Canada, [4]Department of Medical Genetics; University of British Columbia; C201 – 4500 Oak Street, Vancouver, BC, V6H 3N1; Canada,

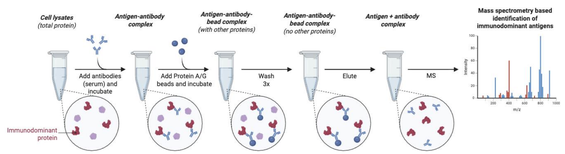
BACKGROUND

- *Fusobacterium nucleatum (Fn)* is a gram-negative anaerobic bacterium found in the oral cavity[1]
- Oral microinjuries enable hematogenous spread to distant tissues[2]
- *Fn* acts as an opportunistic pathogen by colonizing tumors such as colorectal cancer (CRC)[2]
- Tumor colonization is mediated by adhesins such as **Fap2, RadD, and FadA** which also promote immune suppression, tumor progression, and immune evasion[1,3]
- **To date, there is no way to selectively block *Fn* tumor colonization**
- Since antibiotics cannot be used to selectively prevent *Fn* tumor colonization, there is a need for targeted, durable immunotherapeutic approaches, such as vaccines[2]
- **Immunodominant proteins can be strong candidates for vaccine antigens**



METHODS

1. Serum samples were collected from vaccinated (prime/boost with O2-killed Fn via IM injection) or unvaccinated mice, and CRC patients and healthy controls (HC)
2. *Fn* was cultured under anaerobic conditions, and lysates were prepared by sonication
3. Immunoprecipitation (IP) isolated immunodominant antigens by combining *Fn* lysate and serum antibodies



4. IP's were validated using SDS-PAGE / Western blot
5. Identified bound proteins by mass spectrometry (MS)

Fn autotransporters are dominant antibody targets in vaccinated mice and are also detected in CRC patients and healthy controls

IP-MS Analyses Reveal Enrichment of Predicted *Fn* Antigens in Pooled O_2-Killed *Fn* Vaccinated Mouse Serum

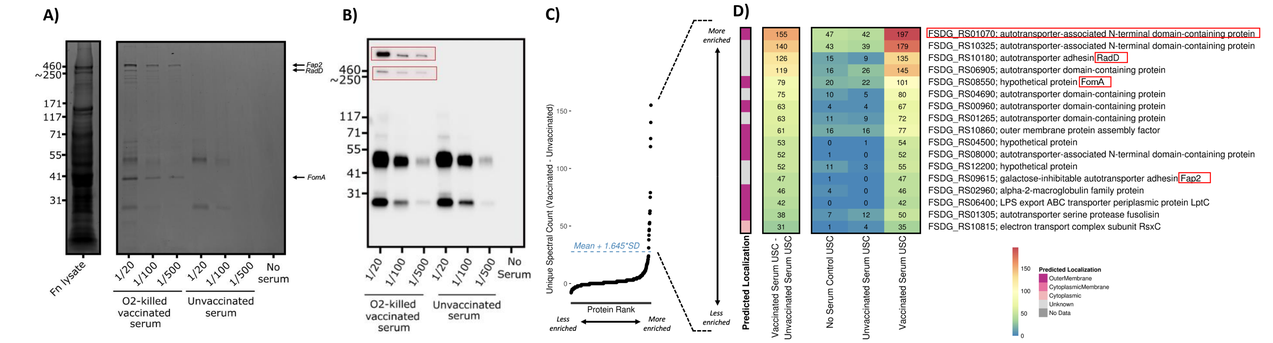


Figure 1 | Immunoprecipitation Analyses Validating *Fn* Antigen Enrichment across pooled O_2 killed *Fn* Vaccinated, Unvaccinated, and No Serum Mouse Models
A) SDS-PAGE analysis comparison of serum input volumes (1:20, 1:100, 1:500 dilutions respectively). **B)** Western Blot validation of Fap2 antigen using a custom made anti-Fap2 monoclonal mouse antibody. **C)** Ranked protein enrichment curve showing IP-MS derived protein enrichment (vaccinated − unvaccinated); cutoff at mean + 1.645 × SD, representing the top 5% of binders and candidate immunodominant proteins. **D)** Heatmap of the top 5% of IP-MS–enriched proteins (n = 26), defined by a mean + 1.645 × SD cutoff. Predicted localization (pSORTb) is indicated.

RESULTS

- Antibody responses were primarily directed against outer membrane autotransporter proteins
- Identified immunodominant antigens are relevant virulence proteins (Fap2, RadD) and dominant outer membrane (OM) proteins (FomA)
- Identified autotransporters could be effective targets for vaccines or other immunotherapies

IP-MS Analyses Reveal Enrichment of *Fn* Antigens in 4 Most and 4 Least Seroreactive CRC Patients and Pooled HC

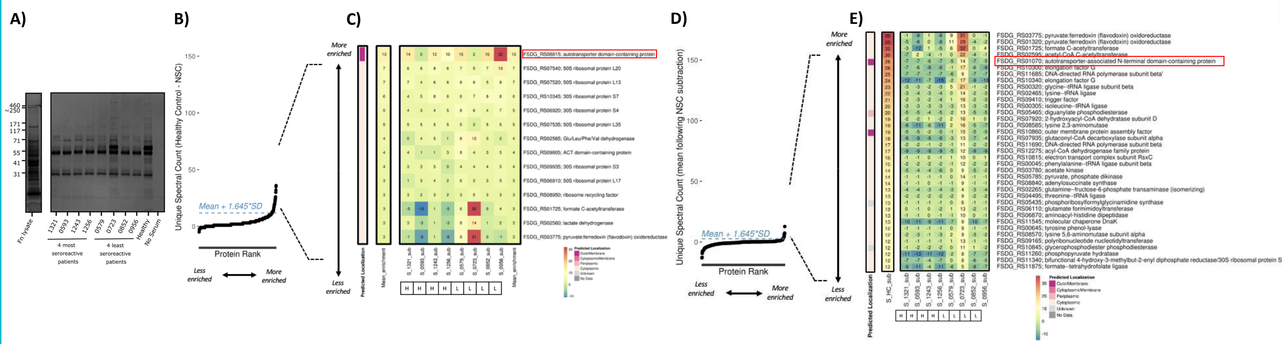
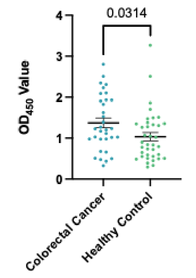


Figure 2 | Immunoprecipitation Analyses Validating *Fn* Antigen Enrichment across 4 Most and 4 Least Seroreactive CRC Patients and Healthy Controls (HC)
A) SDS-PAGE analysis comparison of serum input for the 4 most seroreactive, 4 least seroreactive CRC patient samples and pooled HC. **B)** Ranked protein enrichment curve showing IP-MS derived protein enrichment (CRC − No Serum Control); cutoff at mean + 1.645 × SD, representing the top 5% of binders and candidate immunodominant proteins in CRC patients. **C)** Heatmap of the top 5% of IP-MS enriched proteins (n = 14) for CRC patients, defined by a mean + 1.645 × SD cutoff. Predicted localization (pSORTb) is indicated. **D)** Ranked protein enrichment curve showing IP-MS derived protein enrichment (HC− No Serum Control); cutoff at mean + 1.645 × SD, representing the top 5% of binders and candidate immunodominant proteins in HC. **E)** Heatmap of the top 5% of IP-MS enriched proteins (n = 37) for HC patients, defined by a mean + 1.645 × SD cutoff. Predicted localization (pSORTb) is indicated.

RESULTS

- The most immunodominant antigen in CRC patients is an outer membrane autotransporter protein
- A top immunodominant protein in healthy controls is the most immunodominant protein in vaccinated mice
- There does not seem to be a significant difference in responses to *Fn* between high vs low serology samples

RESULTS



- There is a significantly elevated response against *Fn* antigens in CRC patients compared to HC

Supplementary Figure | ELISA-based serological screening of CRC patients using *Fn* (7-1) lysate to identify high and low seroreactive individuals
Top 4 most and bottom 4 least CRC patients were selected for further IP-MS analyses (Figure 2)

CONCLUSIONS & FUTURE DIRECTIONS

- IP-MS identified immunodominant *Fn* antigens primarily as OM autotransporter proteins in vaccinated mice
- Key virulence factors (RadD, Fap2, FomA) were strongly enriched in vaccinated mice, suggesting potential for vaccine mediated protection
- Similar OM autotransporter proteins were detected in CRC patients and HC, however responses were more varied compared to vaccinated mice
  - Strong enrichment of intracellular proteins (e.g. ribosomal proteins) suggest potential cross-reactive binding
- Both high and low seroreactive patients targeted similar antigens
  - This suggests differences in response strength rather than the antigens being recognized
- Future work will assess strain-specific and potential cross-reactive responses (*Fn* strains 7-1 vs 23726)
- Comparisons of antibody responses between CRC patients with high and low *Fn* tumor load will be performed
- Identified proteins will be evaluated as vaccine or immunotherapy targets

Petra Jozsa, petra_jozsa@sfu.ca

REFERENCES

1. Liu et al. J. Proteomics **195** (2019) 2. Holt RA. Cell Host & Microbe. **1** (2023) 3. Zhang et al. Nat Microbiol. **9** (2024)

ACKNOWLEDGEMENTS

BC CANCER · GlycoNet · undergraduate research symposium · SFU SIMON FRASER UNIVERSITY