Genetic Modifiers and the Gut Microbiota in Colorectal Cancer Development

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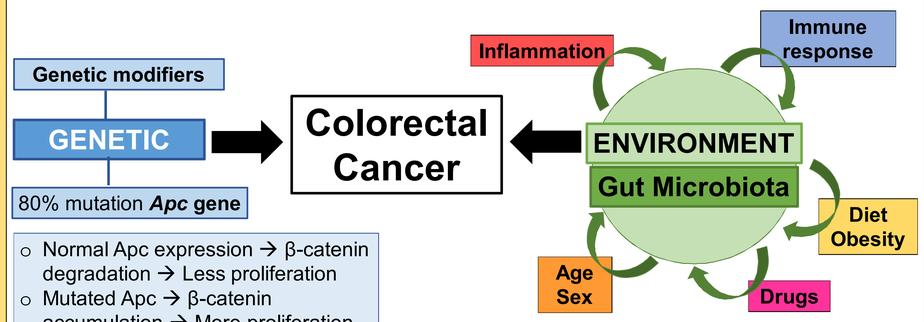
Background

- Colorectal cancer (CRC) is a multifactorial disease induced by multiple genetic and environmental factors.
- Genetic modifiers connect the central role of *Apc* to the myriad of factors implicated in CRC risk, including the gut microbiota (GM)



- Mouse model: C57BL/6-*Apc*^{Min}
  B6

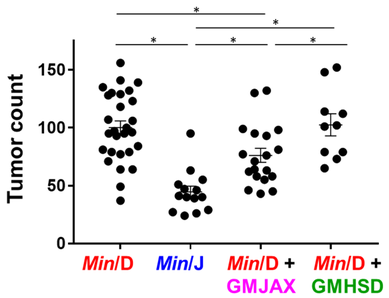


Figure 1. Genetic and GM modifiers of *Apc*^{Min} phenotype. Genetic divergence between B6/J and B6/D resulted in spontaneous modifiers of the *Apc*^{Min} tumor phenotype in the B6/J genome. Rederivation with different complex GM also demonstrated an environmental effect of the GM on tumor phenotype.

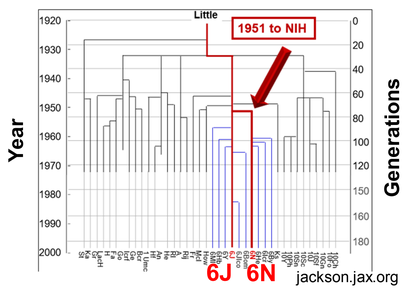
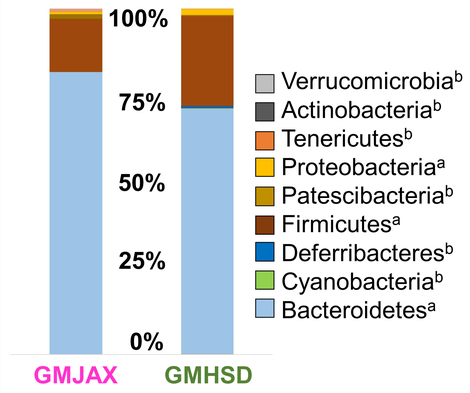
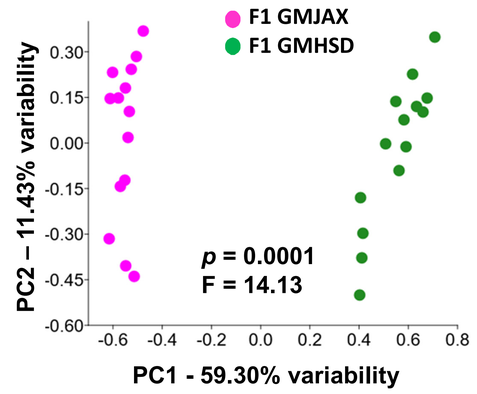


Figure 2. Genetic divergence between B6/J and B6/N. Genetic differences between B6J and B6N background substrains confer phenotypic changes in **metabolism and immunology.** Previously characterized phenotypic differences include glucose homeostasis, weight gain, and insulin resistance. It is unclear whether genetic modifiers in these substrains differentially modify the *Apc*^{Min} tumor phenotype.

Hypothesis: Genetic modifiers present in the wild-type B6NHsd genome will reduce the tumor number compared with the B6D-*Apc*^{Min} inbred strain. GM colonization will also affect tumor multiplicity in B6NB6DF1 hybrids.

B6NB6DF1 Gut Microbiota Composition





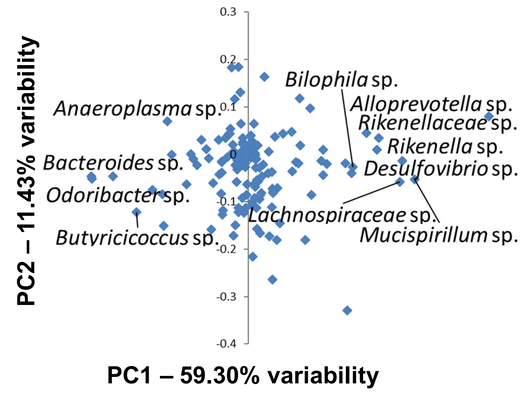
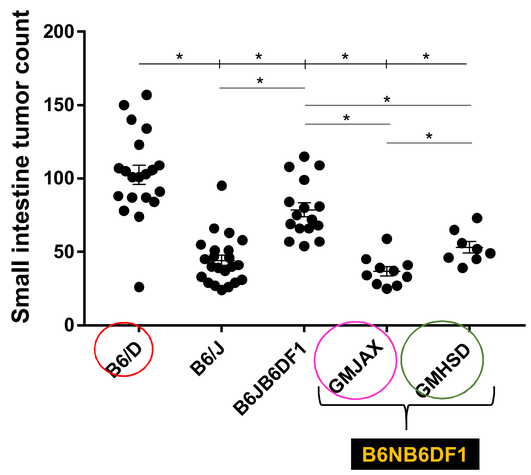


Figure 4. . Mean relative abundance of phyla are indicated by bar graphs (left). Mean relative abundance of all listed phyla were significantly different between GMJAX and GMHSD, as determined using Student's t-test (a) and Mann-whitney Rank sum test (b) for non-normally distributed data. **Principal component analysis (PCA) and loadings plot comparing the GM (OTU level) of B6NB6DF1 mice rederived with either GMJAX or GMHSD.** PCA (center) represents the overall variation of complex GM between groups. Loadings plot (right) indicates OTU populations contributing the most variation between GMJAX and GMHSD. Statistical significance was determined using one-way PERMANOVA and Bray-Curtis dissimilarity index, $p < 0.05$

GM and Genetic Modifiers of Min in the B6N Strain



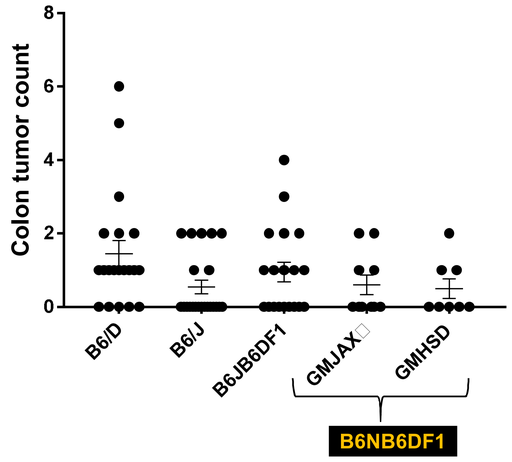
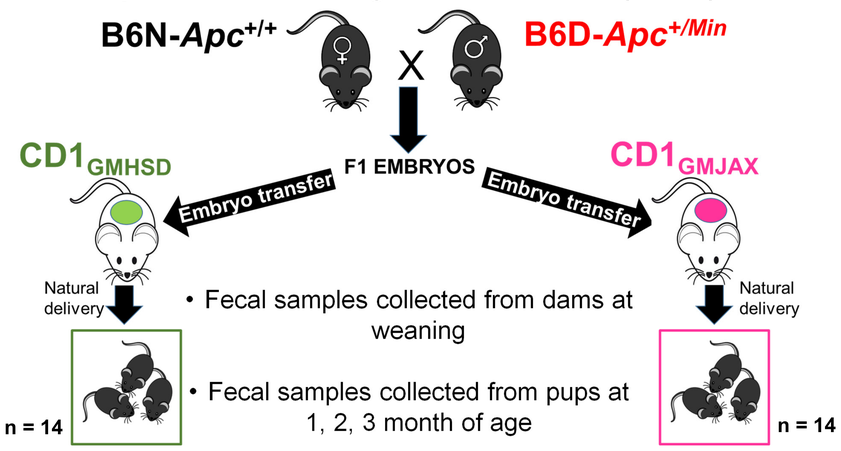


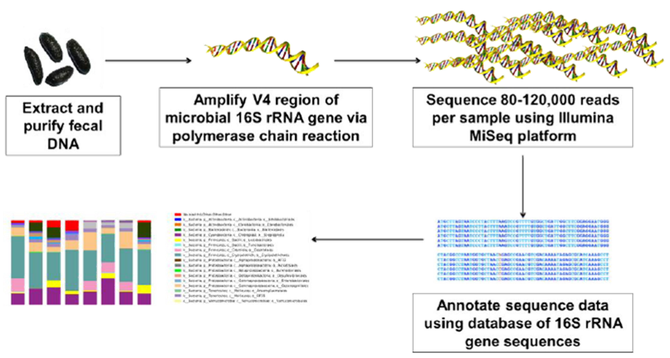
Figure 5. Small intestine (left) and colonic (right) tumor counts of *Apc*^{Min} mice. Terminal SI and colonic tumor counts following sacrifice at 3 months of age. B6NB6DF1-*Apc*^{Min} mice had significantly fewer small intestinal tumors than both the B6D-*Apc*^{Min} parent line and the B6JB6DF1 hybrid animals. Statistical significance was determined using ANOVA.
GMJAX had significantly fewer small intestinal tumors than GMHSD. Statistical significance was determined using Student's t-test. No statistical differences were noted in colonic tumor counts. Asterisks indicate statistical significance between groups, $p < 0.05$ was considered significant.

Methods

A. Complex Microbiota Targeted Rederivation (CMTR)



B. Characterization of the gut microbiota



C. Assessment of disease severity: Terminal tumors counted in small intestine and colon.

Figure 3. A. Complex Microbiota Targeted Rederivation (CMTR): B6NB6DF1 embryos generated from a wild-type B6NHsd female crossed with a B6D-*Apc*$^{+/Min}$ male were implanted into surrogate dams that harbored distinct complex microbiota profiles that originated from the Jackson Laboratory (GMJAX) or Envigo (GMHSD). **B. Characterization of the gut microbiota:** fecal samples collected from dams at weaning and from pups at 1, 2 and 3 months of age were used to extract and purify fecal DNA. The V4 region of microbial 16S rRNA gene was amplified via PCR and sequenced and compared to SILVA database to define the GM bacterial composition. **C. Assessment of disease severity:** mice were sacrificed at 3 months of age, and small intestine and colonic tumors counted.

Conclusions and Future Directions

- The B6N background strain has dominant genetic modifier(s) of the Min phenotype.
- The tumor repression effect is significantly stronger in the B6N strain compared to B6J background strain.
- GM and genetic modifiers of Min can significantly impact tumor counts in *Apc*^{Min} studies, and may contribute substantially to reproducibility across studies.
- These data will lead to further studies in identifying and characterizing specific genetic and GM modifiers that may have relevance to human CRC pathogenesis.

Acknowledgments

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