Multi-generational effect of western diet on colorectal cancer and impact of green tea on cancer prevention

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BACKGROUND

- A calorie-dense, nutrient-poor Western diet is associated with increased risk of colorectal cancer and mortality in the U.S.
- Animal model studies to identify functional foods for cancer prevention generally do not take into account typical inappropriate micronutrient intakes for Americans.
- Mounting evidence points to the critical influence of maternal diet on disease risk in offspring, including cancer, obesity and diabetes, as well as to the potential benefit of maternal diet modification for disease prevention.
- The impact of typical U.S. nutrition on colon cancer risk across multiple generations of offspring has not been investigated, nor are the mechanisms(s) by which diet may influence colon cancer risk in subsequent generations understood.

OBJECTIVES & HYPOTHESIS

Objectives
- Determine whether multi-generational or ancestral exposure to the typical U.S. diet increases risk of colorectal cancer in offspring.
- Determine the efficacy of green tea for cancer prevention in a murine model of inflammation-associated colorectal carcinogenesis using a basal diet that represents typical U.S. nutrition with respect to macro- and micronutrient intakes.

Hypotheses
- Ancestral exposure via a parental diet that reflects typical U.S. nutrition will increase risk of colon cancer in offspring.
- Green tea will have a substantially greater health benefit in mice exposed to a Westernized diet.

PRELIMINARY CONCLUSIONS

- Analyses of cancer outcome complete for F2 generation mice provided water. Major conclusions:
  - Ancestral exposure to TWD markedly increased CRC incidence and severity in F2 offspring that were never fed this diet.
  - Exposure to TWD over multiple generations had an even stronger effect on CRC severity (more tumors, larger tumors, increased tumor burden) in F2 offspring compared to mice directly fed TWD.
  - Body weight and composition (statistics not yet complete):
    - Body weight in F2 male offspring exposed to DIO diet over multiple generations was significantly greater than those exposed during a single generation, either direct or ancestral.
    - TWD and DIO diets both promoted increased fat mass in F2 offspring, though females appear less responsive to TWD and more responsive to DIO fed over multiple generations.
  - Ancestral exposure to either TWD or DIO diet did not appear to markedly alter body weight or composition in F2 offspring.

>12k data points collected! Data curation ongoing.
- Other ongoing work includes assessment of patterns of gene promoter DNA methylation and transcript abundance for a panel of cancer critical genes in colon tissues.

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DESIGN

Experimental diets and groups
- Mice were fed test diets according to their group assignment shown to the left during breeding, weaning and grow out periods.
- F3 generation offspring allocated to four subgroups:
  - Sham saline injection with water
  - AOM+DSS injection with water
  - AOM injection with green tea supplementation
  - AOM+DSS injection with green tea supplementation

Endpoints across generations
- Food and water consumption (to estimate energy and green tea intakes)
- Body weight at weaning, weekly body weight gain, and final body weight
- Breeding success, cannibalism rate, litter size, and birth weight
- Fasting glucose and glucose tolerance
- Body composition by EchoMRI

Terminal endpoints in F3 offspring
- Colon tumor incidence, multiplicity, size, and burden
- Estrus cycling in adult females
- Biomarkers of gut inflammation
- Targeted gene expression analysis of colon mucosa by multiplex qPCR
- Targeted DNA methylation analysis of colon mucosa by Ion Torrent ultra-deep bisulfite amplicon sequencing (Apc, Tm1, Cdh1, Cdk2a2, Mgmt, Mth1, Rarb, Rara)

OBSERVATIONS

- Figure 1. Ancestral exposure to the TWD markedly increased CRC incidence and exacerbated disease severity in F2 offspring, whereas a simple high fat diet was mostly without significant effect.

- Figure 2. Body weight and composition across all generations (preliminary)

Fig. 1. Data shown are incidence or mean ± SEM (n=8–16 for tumor multiplicity, average tumor volume and tumor burden in F1 generation offspring fed experimental diets outlined in Fig 1 and provided plain drinking water. (Data for mice provided green tea extract are still being tabulated.) For panels A and B, different letters indicate groups that are statistically different by Fisher’s Exact test, with Bonferroni adjustment for multiple testing. For all other panels, different letters indicate groups that are statistically different (p<0.05) according to fit model ANOVA with Tukey HSD post-hoc tests and cage as a randomized nested factor within diet in the statistical model (JMP v.13, SAS Institute).

Fig. 2. Data shown are the mean final body weight and the percent mass as fat as determined by EchoMRI + SEM of 24 week-old mice after breeding had completed for each generation (F2 to F4 generations, no breeding of F1 mice). Body composition was determined by EchoMRI. Note that data are merged across generations when comparing sex differences that have not varied across the generations. For example, two groups of F2 mice were used for ANOVA and two were fed TWD; thus the top left figure shows just two bars for males and females. Values are mean ± SEM. Data for mice provided green tea extract in F2 generation are still being tabulated. Preliminary statistical analyses are shown for F1 generation mice (n=16 to 24 mice for each group, within each sex). Different letters indicate groups that are statistically different (p<0.05) according to fit model ANOVA with Tukey HSD post-hoc tests and cage as a random nested factor within diet in the statistical model (JMP v.13, SAS Institute). Statistical analyses have not been performed for generations F3 through F5 or to compare outcomes across all generations.