Gut microbial dysbiosis associates with the early-onset breast cancer in BRCA mutation carriers

Po-Han Lin, Ya-Hui Chen, Tzu-Chun Yen, Hao-Ting Chang, Yuan-Chia Chu, Ziy-Uan Liu, Sung-hsin Kuo, Chiun-Sheng Huang
National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan

Abstract

Germline BRCA mutation carriers have a high lifetime risk of breast cancer, yet onset age varies widely. Gut microbiota influence host immunity and metabolism, and dysbiosis has been linked to carcinogenesis. We investigated whether specific microbes contribute to early breast cancer in BRCA carriers.

We first analyzed fecal samples from 27 carriers with early-onset breast cancer (<40 years) and 8 older cancer-free carriers (>60 years) using full-length 16S rRNA sequencing. LEfSe analysis identified Eggerthella lenta (E. lenta) and Dorea longicatena as significantly enriched in early-onset cases. Because these microbes are typically associated with aging, their presence in young BRCA carriers suggests cancer relevance beyond age.

We next studied 102 carriers (54 BRCA1, 48 BRCA2); 64 had breast cancer. Carriers with E. lenta developed breast cancer earlier than those without it (median 42.2 vs. 52.8 years, p<0.001), a trend observed in both BRCA1 and BRCA2 subgroups.
In brca1-null mice, colonization with E. lenta accelerated mammary tumor formation. Metabolomic profiling of human urine and mouse serum showed elevated tryptophan–kynurenine pathway metabolites associated with E. lenta. Quinolinic acid, a downstream metabolite, promoted earlier tumor onset in mice and induced replication stress and genomic instability in BRCA1-mutant mammary cells.

These findings reveal a gut microbe–metabolite axis that may drive early-onset breast cancer in BRCA mutation carriers.

Introduction

1. Germline BRCA mutation carriers face a high risk of developing breast cancer, yet individual risk and age of onset vary significantly, influenced by both genetic and environmental factors.
2. The gut microbiota is crucial for host health, regulating immunity, metabolism, and endocrine functions. Dysbiosis—an imbalance of gut microbiota—has been linked to diseases including cancer.
3. We aimed to investigate whether specific gut microbes influence breast cancer risk in BRCA mutation carriers and to explore the underlying mechanisms.

Methods and Materials

1. 16s full length sequencing: Fecal materials were collected from mutation carriers and be frozen at -80°C. DNA extraction was performed by using Qiagen DNA extraction kit based on the protocol. Microbiome full length 16S rRNA DNA was amplified by LoopSeq™ 16S Microbiome 24-Plex Kit.
2. Metabolites analysis: urine samples were analyzed by GC-MS.
3. In vitro study: Human BRCA1 (185delAG/+) MCF10A Cell Line was used for validation of the effect of metabolite.
4. Animal model: A Wap-Cre brca1$^{flox/flox}$; p53$^{flox/flox}$ mouse model was treated with 4 weeks of antibiotics (ampicillin, neomycin sulfate, metronidazole, and vancomycin) to generate antibiotic-induced microbiome depletion (AIMD) model. The AIMD mice received consecutively feeding for 14 days of the target microbe, then followed with weekly feeding of the target microbe.

Results

1. First, we studied 27 BRCA mutation carriers with early-onset breast cancer (<40 years old) and 8 old carriers (>60 years) without cancer. The linear discriminant analysis (LDA) effect size (LEfSe) analysis showed that the *Eggerthella lenta* (*E. lenta*) and *Dorea longicatena* (*D. longicatena*) were the most significantly enriched bacteria in feces of the mutation carriers with early-onset breast cancer, compared to those in the old carriers without breast cancer.

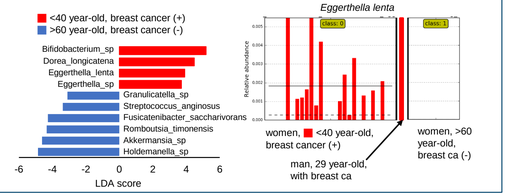


Figure 1. LDA score and the phylogenetic tree

Results

2. Second, we expanded the study to 102 BRCA mutation carriers (54 BRCA1, 48 BRCA2), 64 of whom had breast cancer. Mutation carriers with fecal E. lenta had a significantly earlier age of breast cancer than those without it (median age: 42.2 vs. 52.8 years-old, p<0.001), while D. longicatena showed no significant association.
3. Subgroup analyses by genotype confirmed that E. lenta was significantly enriched in both young affected BRCA1 and BRCA2 mutation carriers (both p<0.01), compared to that in the old unaffected BRCA1 and BRCA2 mutation carriers

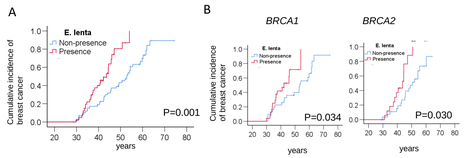


Figure 2. cumulative incidence of breast cancer in the entire cohort and BRCA1/2 subgroup

4. The AIMD mice were gavaged with phosphate-buffered saline or E. lenta for consecutive 2 weeks and followed by once per week (Figure 3A). The brca1 null mice gavaged with E. lenta developed breast tumors significantly earlier than those without E. lenta (p=0.022, Figure 3B and 3C). Pathology revealed high-grade carcinoma with high mitosis, similar to human breast carcinoma.

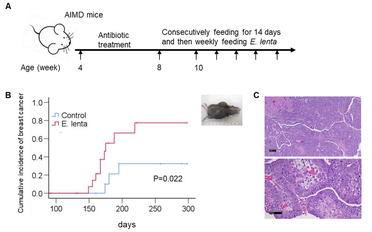


Figure 3. the AIMD model generated from brca1 null mice. These mice with E. lenta feeding developed breast cancer earlier than those with E. lenta feeding.

Results

5. Untargeted metabolomics on the urine of the young affected (< 40 years-old) and old unaffected (> 60 years-old) mutation carriers. Metabolite Variable Importance in Projection (VIP) score revealed the significant enrichment of tryptophan and its downstream multiple metabolites in the young affected mutation carriers, including Kynurenine, 3-hydroxykynurenine, 3-Hydroxyanthranilic acid, picolinic acid and quinolinic acid.

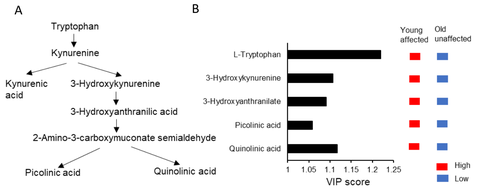


Figure 4. (A) Metabolism pathway of tryptophan (B) VIP score of the metabolites

6. Quinolinic acid was intraperitoneal injected in the brca1 null mice. The breast cancer developed significantly earlier in the mice injected with quinolinic acid than those without quinolinic acid injection (p<0.01).

7. DNA fiber assay revealed significantly increased replication speed and impaired replication fork protection in the BRCA1 (185delAG/+) MCF10A cell line with co-culture of 10uM quinolinic acid, compared to those without quinolinic acid co-culture.

Conclusions

Our study demonstrated that dysbiosis is associated with early-onset breast cancer in BRCA mutation carriers. The E. lenta-derived tryptophan/kynurenine/quinolinic acid pathway induces the replication stress and genomic instability that drive breast cancer.

.

Contact

<your name> Po-Han Lin
<your organization> National Taiwan University Hospital
Email: pohanlin01@ntu.edu.tw
Website: https://www.mc.ntu.edu.tw/medgenpro/Vcard.action?q_type=A02&q_itemCode=742&l=en_US

This presentation is the intellectual property of the author/presenter. Contact them at <<your email address>> for permission to reprint and/or distribute.

© Poster Template by Genigraphics® 1.800.790.4001 www.genigraphics.com