Integrative 16S Metagenomic Profiling and Structure–Dynamics–Based Inhibitor Discovery Targeting ClbP in Breast Tumour Microbiomes

Thiyonila Berchmans and Jeyakanthan Jeyaraman*

Department of Bioinformatics, Science Block, Alagappa University,
Karaikudi, 630 003.

(E.mail: thiyonila@gmail.com)

Abstract

The tumour microbiome is becoming a notable participant in cancer biology, and this is attributed to the involvement of microbial genotoxins that can cause host DNA damage and create pro-tumorigenic microenvironments. Colibactin, a polyketide-nonribosomal peptide genotoxin of *Escherichia coli* and other *Enterobacteriaceae*-coded by the pks genomic island, is one of the key microbial carcinogens in which there is strong evidence of association with colorectal cancer and with which there is growing evidence of association with breast tumour tissues. Recent microbiome research demonstrates that breast tumours may have pks-positive bacteria, which hints at a possible role of colibactin-mediated genotoxic stress in breast cancer development. The membrane-anchored serine hydrolase ClbP is essential in the processes of colibactin maturation, which cleaves the N-acyl-D-Asn prodrug motif of precolibactins into the active DNA-crosslinking metabolites. We combine 16S rRNA gene sequencing of breast tumour and the control tissues with a structure-based drug discovery pipeline and assess ClbP as a drugable anti-virulence target in this study. Taxonomic evidence in the taxonomic enrichment of *Escherichia*, *Klebsiella* and *Enterobacter* in tumour samples gives support in the occurrence of potential pks-harbouring colibactin-producing bacteria. We have conducted high-throughput virtual screening of repurposing libraries on the basis of available ClbP crystal structures (PDB: 4E6W, 4E6X), after which MM-GBSA rescoring, DFT refinement, redocking known boronic-acid inhibitors, and 500-ns long molecular dynamics simulations were conducted. The best of them showed consistent contact with the major catalytic residues (Ser95, Lys98, Tyr186) and the D-Asn recognition pocket, indicating that they could prevent colibactin maturation. Combined with the current results, ClbP inhibition is a suggested microbiome-based therapeutic approach that can alleviate genotoxic pressure in breast cancer without disrupting the shape of the microbial community. The dual-compete microbiome-to-molecule system that we have developed provides a basis of new anti-virulence therapy against tumour-associated bacteria.

Keywords: Tumor-associated microbiome, Colibactin biosynthesis, ClbP inhibition, 16S rRNA metagenomics, Microbiome-targeted therapy.