**SN-38G metabotyping predicts irinotecan toxicity via gut microbial β-glucuronidases in colorectal cancer**

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**Background and aims.** Irinotecan (IRT)-induced gastrointestinal toxicity in colorectal cancer (CRC) patients is driven by gut microbial β-glucuronidase (GUS) reactivation of SN-38G, yet interindividual determinants remain uncharacterized. We hypothesized that SN-38G metabotyping identifies patients at risk via GUS-harboring bacteria. This study aims to define fecal SN-38G metabotypes in CRC, validate causal roles of EM-enriched microbes/GUSs in IRT toxicity, and characterize microbe-metabolite-pathway perturbations underlying metabotype divergence.

**Methods.** Fecal SN-38G hydrolysis activity stratified 90 CRC patients into poor (PM), moderate (MM), and extensive metabolizers (EM). Fecal microbiota transplantation (FMT) from EM/PM patients to IRT-treated mice (n=32/group) assessed toxicity causality. Metagenomics (20 EM/20 PM) and urinary glucuronide profiling (LC-MS/MS; 20 EM/17 PM) identified differential taxa/metabolites. Mono-colonization with EM-enriched species *Faecalibacterium prausnitzii* (*Fp*) in absence/presence of GUS inhibitor amoxapine tested mechanistic contributions.

**Results.** EM patients (27.8%) exhibited advanced TNM stage and distinct microbiomes enriched in 26 GUS-harboring species (e.g., *Fp*, *Alistipes shahii)* and 17 GlcA-utilizing taxa. EM-FMT caused 46.9% mortality vs. 0% in PM-FMT, with severe diarrhea and intestinal SN-38 accumulation. Mono-colonization with *Fp* recapitulated toxicity (16.7% mortality), abrogated by AMX (100% survival). EM microbiomes harbored more GUS genes (1,705 vs. 1,414), dominated by *Fp* GUSs (21 isoforms). Urinary metabolomics revealed EM enrichment of flavonoid glucuronides (e.g., dihydroxy-1H-indole) and depletion of organic oxygen conjugates (e.g., dihydro-3-coumaric acid), correlating with suppressed pentose/GlcA interconversions (xylB, uxaC).

**Conclusion/Discussion.** SN-38G metabotyping stratifies CRC patients by IRT toxicity risk via EM-enriched GUS consortia. Our findings directly support metabotyping as predictive biomarker for precision dosing. *In vitro* SN-38G hydrolysis assays enable toxicity screening without rodent models, while GUS inhibitors (e.g., amoxapine) can be used as adjuvants for IRT regimens to mitigate toxicity in vulnerable metabotypes.

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