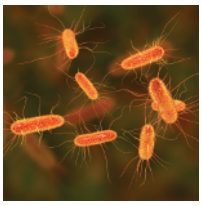
Microbiome

Major Finding: The *pks*$^+$ *E. coli* metabolite colibactin caused a unique mutational signature in intestinal organoids.

Concept: Colibactin had been linked to colorectal cancer, and this signature was seen in patient colorectal cancers.

Impact: This shows colibactin's carcinogenic mechanism and strengthens the link between *pks*$^+$ *E. coli* and colorectal cancer.

COLIBACTIN CAUSES COLORECTAL CANCER–ASSOCIATED MUTATIONAL SIGNATURE



Colibactin, an *Escherichia coli* metabolite that has been linked with colorectal cancer development, putatively exerts mutagenic effects via DNA alkylation and induction of double-strand breaks (DSB). *E. coli* harboring the pathogenicity island *pks* (also called *clb*), encoding the enzymes that produce colibactin, are found in approximately 20% of healthy individuals, 40% of people with inflammatory bowel disease, and 60% of people with familial adenomatous polyposis or colorectal cancer. To identify the mutational consequences of *pks*$^+$ *E. coli* exposure, Pleguezuelos-Manzano, Puschhof, Huber, and colleagues microinjected a *pks*$^+$ *E. coli* strain obtained from a colorectal cancer biopsy into the lumens of clonal human intestinal organoids. Compared with organoids injected with an isogenic negative-control strain incapable of producing colibactin, organoids exposed to *pks*$^+$ *E. coli* exhibited DSBs and interstrand cross-links, verifying that *pks*$^+$ *E. coli* caused DNA damage in this model. Organoids were then grown from single cells, enabling five-month exposure to *pks*$^+$ or control *E. coli*, and subclones from these organoids were used to establish new organoids, which were subjected to whole-genome sequencing. Organoids grown from those exposed to *pks*$^+$ *E. coli* had higher levels of single-base substitutions, predominantly T-to-N substitutions, which were preferentially found in the middle of ATA, ATT, and TTT nucleotide triplets. *pks*$^+$ *E. coli*–exposed organoids also had a unique indel signature defined by single-T deletions at T homopolymers, defining—together with the single-base substitutions—a *pks*$^+$ *E. coli*–induced mutational signature. The mutations caused by the *pks*$^+$ *E. coli* showed a transcriptional-strand bias, which indicated the repair of damaged adenosines by transcription-coupled nucleotide-excision repair and confirmed that colibactin damages DNA by binding this base. Importantly, the *pks*$^+$ *E. coli*–induced mutational signature was enriched in samples from colorectal cancer metastases compared with metastases of other origins, a finding validated in an additional genomic dataset derived mostly from primary tumors. Together, these results not only elucidate the *pks*$^+$ *E. coli*–induced mutational signature, but also build a stronger mechanistic link between exposure to these colibactin-producing bacteria and colorectal cancer. ■

Pleguezuelos-Manzano C, Puschhof J, Huber AR, van Hoeck A, Wood HM, Nomburg J, et al. Mutational signature in colorectal cancer caused by genotoxic pks$^+$ *E. coli. Nature 2020;580:269–73.*

Drug Discovery

Major Finding: The MaMTH-DS assay detected inhibitors of mutant EGFR in non–small cell lung cancer cells.

Concept: Two inhibitors would not have been identified by standard *in vitro* kinase or cell-based assays.

Impact: MaMTH-DS, which can be adapted for other receptor tyrosine kinases, provided four candidate drugs.

NEW DRUG-DISCOVERY ASSAY IDENTIFIES NOVEL MUTANT-EGFR INHIBITORS

In vitro kinase assays have been successful in identifying inhibitors of cancer-driving mutant receptor tyrosine kinases (RTK) such as mutant EGFR. However, these assays have limitations, including the inability to detect inhibitors that require additional components to mediate their effects and the inability to test for important drug characteristics, such as lack of cellular toxicity. Saraon, Snider, and colleagues describe an adaptation of their previously developed mammalian membrane two-hybrid (MaMTH) assay, originally designed to detect protein–protein interactions between integral membrane proteins in live-cell membranes. In the new assay, termed MaMTH-DS (for MaMTH drug screening), RTKs—which are membrane proteins—are subjected to high-throughput screening for inhibition by small molecules. As a proof of principle, MaMTH-DS was used to test a panel of 2,960 small-molecule candidates for inhibition of an osimertinib-resistant EGFR triple mutant on non–small cell lung cancer (NSCLC) cells. Using this screening method, three new chemicals (midostaurin, AZD7762, and EMI1) emerged as inhibitors of mutant but not wild-type EGFR, and one more candidate compound (gilteritinib) was identified based on functional similarity to one of the inhibitors. Biochemical assays and experiments using EGFR-mutant NSCLC cell lines and organoids validated that these compounds were potent and specific. These results imply that midostaurin and gilteritinib, which were recently approved by the FDA to treat FLT3-mutant acute myeloid leukemia, may be of use in certain EGFR-mutant NSCLCs. Importantly, AZD7762 and EMI1 would not have been identified as mutant-EGFR inhibitors in commonly used *in vitro* or cell-based assays. Collectively, this work provides new candidate drugs for further investigation and demonstrates the utility of MaMTH-DS as a screening platform—and, notably, the assay could be adapted to identify inhibitors of RTKs other than EGFR as well. ■

Saraon P, Snider J, Kalaidzidis Y, Wybenga-Groot LE, Weiss K, Rai A, et al. A drug discovery platform to identify compounds that inhibit EGFR triple mutants. Nat Chem Biol 2020 Feb 24 [Epub ahead of print].