

Targeting Colibactin, a genotoxic mutagen from *E.coli*, as a driver of genomic instability in prostate cancer metastatic progression.

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Background - Metastatic prostate cancer (mPC) remains lethal due to therapy resistance driven in part by genomic instability. Recent studies implicate *colibactin-producing Escherichia coli* (*E. coli*) in tumorigenesis. *E. coli* strains positive for the *pks* gene can produce the genotoxin, colibactin, which causes double strand breaks. A colibactin DNA mutational signature was recently identified in colorectal cancer organoids which matched the signature in CRC patients implicating colibactin in CRC etiology. We found that colibactin-producing bacteria are prevalent, occurring in 44% (272/620) of men undergoing prostate cancer screening. Additionally, we identified *E. coli* DNA in prostate and metastatic lymph node tissue, potentially exposing metastatic cancer cells to colibactin. The pelvic nodal basin receives drainage from both the rectum and the prostate thus *E. coli* producing colibactin can come in direct contact with prostate cancer that has metastasized to the pelvic lymph nodes.

Hypothesis - Cancer cells are more prone to genomic instability than non-cancerous cells; therefore, exposure of prostate cancer metastases in the lymph node to colibactin may increase genomic instability and lead to poorer outcomes and increased therapy resistance. We used our patient derived xenograft 3D organoids to elucidate colibactin-induced genomic alterations and mutational signatures that may, in turn, lead to therapy resistance and metastatic disease progression.

Methods—Prostate cancer metastasis patient-derived xenograft (PDXO) 3D organoids were exposed to 10µM colibactin-742. Live cell cycle analyzed using Fucci fluorescent reporter system and quantified with confocal microscopy. DNA and RNA extracted for whole genome sequencing (WGS) and transcriptomics. DNA damage assessed with gammaH2AX immunostaining comparing colibactin-742 to *E. coli* *pks*+ versus *pks*- strain in co-cultures.

Results - *E. coli* were the most abundant of the top 50 species in a mixed cohort of urine, prostate, and lymph node tissue from shotgun metagenomics. Cell cycle and RNA expression analysis in response to colibactin treatment revealed transient S-phase pause and upregulation of genes involved in chromatin organization and transcriptional regulation, including histone genes (*HIST1H2BN*, *HIST1H3E*, *HIST1H2BG*) as well as *CHMP2A* and *SOX11*, suggesting increased chromatin restructuring in response to DNA damage. WGS analysis revealed that colibactin induced significant increases in DNA amplification of already amplified regions and increased tumor mutational burden (TMB).

Conclusions - Together, these findings indicate that colibactin exposure induces a transient S-phase pause and a transcriptional shift consistent with a DNA damage response and replication stress. Colibactin induced genomic amplification and increased TMB support the hypothesis that colibactin may contribute to genomic instability and tumor adaptation in prostate cancer metastases. Therefore, microbiome-derived genotoxins such as colibactin may contribute to genomic instability in prostate cancer, highlighting a potential link between the microbiome and metastatic disease progression.

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