Gut Microbiome Mediates Ferroptosis Resistance for Colorectal Cancer Development
Ruoxi Zhang, Rui Kang, and Daolin Tang

Colorectal cancer is a prevalent cancer type in the United States, affecting both genders and influenced by genetics and environmental factors. The role of the gut microbiome in colorectal cancer development and therapy response is a burgeoning field of study. A recent study uncovered that trans-3-indoleacrylic acid (IDA), a microbial metabolite from *P. anaerobius*, promotes colorectal cancer by inhibiting ferroptosis, a type of nonapoptotic cell death driven by unrestricted lipid peroxidation and subsequent membrane damage. IDA activates aryl hydrocarbon receptor (AHR), a nuclear transcription factor, leading to the expression of aldehyde dehydrogenase 1 family member A3 (ALDH1A3). ALDH1A3, known for aldehyde detoxification, also contributes to ferroptosis resistance by generating reduced nicotinamide adenine dinucleotide (NADH), critical for the synthesis of reduced coenzyme Q10 (COQH10), by apoptosis-inducing factor mitochondria-associated 2 (AIFM2, also known as FPS1). Knocking out AHR, AIFM2, or ALDH1A3 reverses the inhibitory effect of IDA on ferroptosis and IDA-mediated tumor growth. Significantly, *P. anaerobius* is enriched in patients with colorectal cancer, and supplementing IDA or *P. anaerobius* accelerates colorectal cancer progression in spontaneous or orthotopic mouse models. Taken together, these findings suggest that targeting *P. anaerobius*-mediated ferroptosis resistance emerges as a promising strategy to combat colorectal cancer development.

Colorectal cancer ranks as the third most commonly diagnosed cancer and the third leading cause of cancer-related mortality in both men and women in the United States. The development of colorectal cancer is influenced by a multitude of factors, encompassing intrinsic gene alterations, such as adenomatous polyposis coli (APC) mutation, as well as extrinsic elements from the microenvironment. Notably, the role of the gut microbiome in colorectal cancer has become a subject of active research, with our understanding continuously evolving. While the precise mechanisms involved are intricate and not yet fully comprehended, conventional perspectives implicate certain bacteria in triggering inflammatory responses, impacting immune reactions, generating metabolites that can either promote or inhibit cancer development, and even inducing DNA damage and mutations (1).

A recent study conducted by Cui and colleagues has shed new light on this area by suggesting that a tryptophan metabolite derived from *P. anaerobius*, known as trans-3-indoleacrylic acid (IDA), plays a role in promoting colorectal carcinogenesis through the inhibition of ferroptosis (2).

Ferroptosis was initially identified as a RAS-dependent cell death mechanism triggered by experimental small molecules, such as erastin and RSL3. Subsequent research has demonstrated that ferroptosis plays a significant role in both RAS wild-type and mutated tumors. While the molecular mechanisms are context-dependent, dysfunction in antioxidant systems is a hallmark of ferroptotic induction. In addition to the master antioxidant enzyme glutathione peroxidase 4 (GPX4), several GPX4-independent antioxidant proteins, including apoptosis-inducing factor mitochondria-associated 2 (AIFM2/ FPS1), dihydroorotate dehydrogenase (DHODH), and GTP cyclohydrolase 1 (GCH1), contribute to the inhibition of lipid peroxidation in ferroptosis (3). These genes, encoding both GPX4-dependent and GPX4-independent pathways, are regulated by the transcription factor NFE2 like BZIP transcription factor 2 (NFE2L2/NRF2). Targeting the impaired ferroptosis pathway represents an emerging field in precision oncology (4).

To evaluate the influence of gut microbiota metabolites on ferroptosis, Cui and colleagues screened 350 endogenous metabolites from both human hosts and gut microbes. Their findings revealed that administration of 10 μmol/L IDA, in contrast to other tryptophan metabolites, inhibited ferroptosis induced by RSL3 or the imidazole ketone erastin. IDA also promoted tumor growth and progression in both *in vitro* experiments and xenograft mouse models. IDA did not act as a radical-trapping antioxidant to reduce lipid peroxidation. This prompted further investigation into whether the aryl hydrocarbon receptor (AHR), a known transcription factor activated by tryptophan metabolites, played a role in mediating its antiferroptotic effects. As expected, IDA interacted with AHR and induced the expression of the classical AHR target genes, such as cytochrome p450 family 1 subfamily A member 1 (CYP1A1). Subsequently, the authors showed that the absence of AIFM2, as opposed to the absence of GPX4, DHODH, GCH1, or NFE2L2, impeded the antiferroptotic and tumor-promoting effects of IDA in both *in vitro* and *in vivo* settings. This underscores the essential role of AIFM2 in mediating the antiferroptotic effect of IDA.

While the authors did not rule out the potential impact of CYP1A1 (a monooxygenase that generates reactive oxygen species, increasing lipid peroxidation and ferroptosis in intestinal intraepithelial lymphocytes; ref. 5) on AIFM2, their RNA sequencing experiments revealed that IDA upregulated the pathway associated with nicotinamide adenine dinucleotide (NADH) dehydrogenase activity and the gene expression of aldehyde dehydrogenase 1 family member A3 (ALDH1A3). ALDH1A3 is part of the aldehyde dehydrogenase (ALDH) enzyme family, which plays a crucial role in metabolizing aldehydes—potentially toxic organic compounds. Recent studies have demonstrated that ALDH1B1, another member of the ALDH family, can efficiently detoxify 4-hydroxynonenal (4HNE), a byproduct of lipid peroxidation and a mediator of ferroptosis (6). While it was initially assumed that ALDH1A3 would perform a similar role in eliminating metabolic 4HNE, the authors revealed that ALDH1A3 is actually a direct target gene of AHR. This upregulation of ALDH1A3 contributes to ferroptosis resistance by catalyzing the conversion of retinal into NADH, a cofactor for AIFM2’s activity in synthesizing reduced coenzyme Q10 (COQH10; Fig. 1; ref. 7).
patients, primarily attributed to *P. anaerobius*-driven production. Furthermore, both *P. anaerobius* and its supernatant, mediated by AHR, upregulate ALDH1A3 and CYP1A2 levels while inhibiting ferroptosis in vitro. In colorectal cancer mouse models, including the APC<sup>Min</sup>-driven spontaneous model and the azoxymethane/dextran sodium sulfate-induced colitis-associated cancer model, the administration of *P. anaerobius* increased IDA levels. This increase effectively mitigated lipid peroxidation and ferroptosis, ultimately promoting tumor growth. Moreover, clinical tissue analysis indicated increased AHR and ALDH1A3 expression in human colorectal cancer samples, positively associated with adverse clinical outcomes.

In summary, these discoveries not only establish a metabolic mechanism by which changes induced in host cancer cells by gut microbiota metabolites result in resistance to ferroptosis but also have potential implications for the development of preventive strategies for colorectal cancer. However, several questions remain to be addressed in future research. First, it is important to consider that many forms of cell death, including ferroptotic damage, can induce chronic inflammation, which promotes tumorigenesis, rather than inhibits tumor growth. For instance, the conditional knockout of Gpx4 in the pancreas or liver can expedite tumorigenesis via inflammation-associated immune suppression (8, 9). Therefore, it is crucial to investigate the immune microenvironment in the context of this study model. Second, gut microbes serve multiple functions, necessitating precise experimental control to rule out other potential contributors to the observed phenotype. In addition to anti-ferroptotic AHR ligands like IDA, proferroptotic AHR ligands such as L-kynurenine can trigger cell death in natural killer cells, promoting the growth of gastric tumors (10). Third, further evaluation is needed to determine whether conditional knockout of Ahr, Aldh1a3, or Aifm2 in mice has similar effects on spontaneous colorectal cancer development in the presence or absence of *P. anaerobius* or antibiotics.

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