

Claudin-4 as the molecular gateway to *Bacteroides fragilis* toxin-driven oncogenesis: implications for the microbiome–cancer targetome

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Abstract

Enterotoxigenic *Bacteroides fragilis* (ETBF) is among the best-characterised microbial oncobionts implicated in colorectal cancer (CRC), exerting its entire carcinogenic programme through a single zinc-dependent metalloprotease, the *B. fragilis* toxin (BFT; fragilysin). BFT initiates oncogenesis by cleaving the E-cadherin ectodomain on colonic epithelial cells, triggering barrier disruption, mucosal T helper Type 17 inflammation and Wnt/ β -catenin activation. Despite several years of investigation, the epithelial receptor mediating this initiating event remained unknown, constraining both mechanistic understanding and therapeutic development. A landmark study by White et al. resolved this by identifying claudin-4 as the primary BFT receptor via a genome-wide clustered regularly interspaced short palindromic repeat (CRISPR) knockout screen. Receptor binding is not merely permissive but mechanistically instructive. Claudin-4 anchors BFT at the plasma membrane, positioning its active site to access a sterically constrained, membrane-proximal E-cadherin cleavage site inaccessible to a freely diffusing protease. This converges with the tetraspanin-guided substrate positioning described for the mammalian sheddase ADAM10, an unexpected parallel between a bacterially encoded ADAM xenolog and its mammalian counterpart. This structural specificity establishes a potentially druggable interface. Proof-of-concept claudin-4 blockade using a soluble claudin-4 analogue (CLN4sol) attenuates BFT-mediated colonic injury *in vivo*, and the precedent set by zolbetuximab, an approved antibody against the related family member claudin-18.2, substantially de-risks pursuing claudin-4 therapeutically. The claudin-4–BFT interface represents a genuine druggable node at the microbiome–cancer interface, with direct implications for microbiome-informed chemoprevention of CRC.

Keywords: *Bacteroides fragilis* toxin, Claudin-4, Colorectal cancer, Tumour microbiota, Therapeutic targetome, CRISPR screen, Membrane-proximal proteolysis, Oncobiont, Microbiome–cancer interface, Chemoprevention

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The concept of the tumour microbiota, which specific bacteria do not merely colonise but actively drive malignant transformation, has shifted from a provocative hypothesis to established biology over the past two decades. Among the growing cast of microbial oncobionts implicated in colorectal cancer (CRC), enterotoxigenic *Bacteroides fragilis* (ETBF) occupies a singular position. Its entire oncogenic programme is mediated by a single ~21-kDa zinc-dependent metalloprotease, the *B. fragilis* toxin (BFT; fragilysin), making it one of the most tractable systems available for dissecting how a commensal bacterium becomes a carcinogenic agent^[1]. ETBF colonisation is detectable in a substantial proportion of patients with early-stage colorectal neoplasia^[2,3] and drives tumorigenesis in experimental models through BFT-mediated cleavage of the E-cadherin ectodomain on colonic epithelial cells^[4], triggering barrier disruption, mucosal T helper Type 17 inflammation and Wnt/ β -catenin activation in a pro-carcinogenic cascade^[5]. Despite nearly three decades of investigation, the identity of the epithelial receptor through which BFT first engages the colonocyte surface remained unknown^[6,7], a gap that constrained both mechanistic understanding and any rational approach to therapeutic targeting.

A study published in *Nature* in April 2026 by White et al.^[8] closes this gap. Using a genome-wide CRISPR knockout screen, the authors identify claudin-4 as the primary BFT receptor and show that receptor engagement is not merely an anchoring event but is

mechanistically instructive. Claudin-4 recruits BFT to the plasma membrane, enabling it to access a sterically constrained, membrane-proximal substrate. The work resolves a longstanding question in bacterial pathogenesis, defines a druggable interface within the ETBF targetome, and illuminates a new dimension of how commensal bacteria co-opt host epithelial architecture to drive oncogenesis (Fig. 1).

A screen designed for biology

Prior genome-wide clustered regularly interspaced short palindromic repeat (CRISPR) screens for bacterial toxin receptors, including those identifying tissue factor pathway inhibitor (TFPI) as a receptor for *Clostridioides difficile* TcdB^[9] and Frizzled proteins as TcdB co-receptors^[10], have relied on toxin-induced cell death to enrich for resistant knockout populations. BFT does not kill colonocytes, which had historically made this approach inapplicable. White et al.^[8] circumvented this by using the primary measurable consequence of BFT's activity, surface E-cadherin shedding, as their selection readout, isolating BFT-resistant cells that retain surface E-cadherin via fluorescence-activated cell sorting. The reformulation selects directly for the molecular phenotype of interest rather than an indirect surrogate and offers a transferable template for receptor

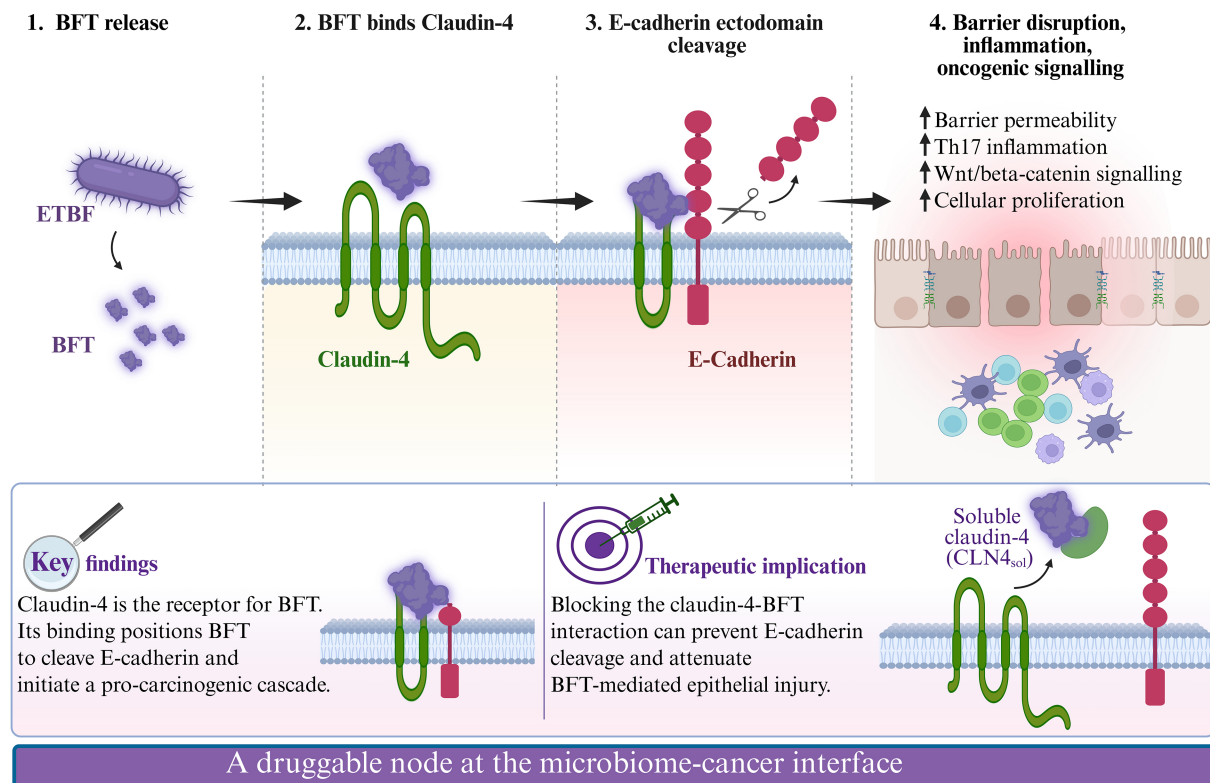


Fig. 1 Claudin-4 is the receptor that enables *B. fragilis* toxin to cleave E-cadherin and drive colorectal oncogenesis. (1) Enterotoxigenic *B. fragilis* (ETBF) secretes the metalloprotease *B. fragilis* toxin (BFT; fragilysin). (2) BFT binds claudin-4 on colonic epithelial cells, positioning its active site at the membrane. (3) This directs BFT to a membrane-proximal but otherwise inaccessible cleavage site in the E-cadherin ectodomain, releasing the soluble E-cadherin ectodomain. (4) Loss of E-cadherin adhesion disrupts barrier integrity and drives a pro-carcinogenic cascade of increased permeability, Th17 inflammation, Wnt/ β -catenin activation and proliferation. Blocking the claudin-4-BFT interaction with the soluble claudin-4 analogue (CLN4_{sol}), prevents E-cadherin cleavage and attenuates BFT-mediated epithelial injury, marking the interface as a druggable node at the microbiome-cancer interface. Created in BioRender. <https://BioRender.com/ibajvho>

discovery across epithelial-targeting bacterial toxins that modify rather than kill their host cells.

Two independent screen replicates converged on claudin-4 as the dominant hit. Validation confirmed that claudin-4 knockout confers approximately 20-fold resistance to BFT-mediated E-cadherin cleavage, rising to approximately 500-fold resistance in claudin-3/claudin-4 double-knockout cells, indicating partial functional redundancy between these highly homologous family members^[11]. Claudin-4's emergence as a receptor for a second distinct bacterial toxin class, alongside its established role as the receptor for *Clostridium perfringens* enterotoxin (CPE)^[11,12], underscores the accessibility of this tight junction protein to luminal pathogens and positions it as a recurring vulnerability at the host-microbiome interface. From a tumour microbiota perspective this is notable. Claudin-4's expression is dysregulated in CRC^[13], raising the possibility that oncogenesis itself may modulate epithelial susceptibility to BFT, a form of microenvironmental feed-forward axis that warrants direct investigation.

A mechanism that reframes how bacteria exploit epithelial architecture

The most conceptually significant contribution of White et al.^[8] is not receptor identification per se but the mechanism it reveals. BFT is a structural xenolog of the mammalian ADAM (a disintegrin and metalloproteinase) family, most likely acquired through horizontal gene transfer from an intestinal host cell^[14]. Yet despite this homology with a class of enzymes that cleave E-cadherin directly, purified

BFT has consistently failed to cleave E-cadherin in cell-free systems, an incongruity that sustained competing mechanistic models for decades. The authors resolved this using a reductionist 293T cell system devoid of endogenous claudins and surface E-cadherin. Co-expression of claudin-4 with E-cadherin reconstitutes full BFT-mediated ectodomain shedding, whereas E-cadherin alone produces no detectable cleavage even at high toxin concentrations. Claudin-4 binding is indispensable for BFT's proteolytic activity at the cell surface.

AlphaFold 3 modelling of the claudin-4-BFT-E-cadherin ternary complex^[15] predicts that claudin-4 anchors BFT at the membrane's surface, directing its active site towards the membrane-proximal linker between E-cadherin extracellular domain 5 and the trans-membrane helix, a site that is inaccessible to a freely diffusing protease. Mutagenesis experiments corroborate this model. Shifting the cleavage site towards the membrane impairs BFT's activity more than equivalent distal deletions, consistent with steric constraints on active-site positioning at the lipid bilayer. This maps directly onto the biology of the mammalian sheddase ADAM10, whose substrate selectivity for N-cadherin and Notch is governed by engagement with specific tetraspanin proteins that provide analogous spatial orientation at the membrane^[16]. The convergence is remarkable: a bacterially encoded ADAM xenolog uses a tight junction protein of the host as a membrane-positioning scaffold and mirrors the tetraspanin-guided mechanism of its mammalian counterpart. This raises broader questions about whether receptor-guided membrane positioning is a general mechanism among gut bacterial metalloproteases.

A single substitution, namely threonine to asparagine at Position 45 within Extracellular Segment 1, the sole residue difference between claudin-4 and claudin-3 at this site, completely abrogates stable BFT binding. Structural characterisation of the native BFT–claudin-4 complex, which the AlphaFold model does not resolve, is the most pressing experimental priority. From the perspective of the tumour microbiota field, understanding how this interface is shaped by the colonic microenvironment, where claudin's expression, tight junction organisation and membrane composition are all altered in neoplastic tissue, will be essential to understanding the full oncogenic relevance of BFT–claudin-4 engagement.

A therapeutic targetome grounded in the microbiome–cancer interface

The therapeutic implications are most compelling when viewed through the lens of the microbiome–cancer field. Despite growing epidemiological and mechanistic evidence for the oncogenic roles of bacteria such as ETBF, *Fusobacterium nucleatum* and *pks+* *Escherichia coli*, the field to date has lacked structurally defined, mechanistically validated drug targets at which microbial oncogenesis can be pharmacologically interrupted. The claudin-4–BFT interface, characterised here with a degree of molecular resolution not previously achieved for any oncobiont–host interaction, fills this gap.

White et al.^[8] demonstrated proof-of-concept for therapeutic claudin-4 blockade using CLN4sol, a computationally designed soluble claudin-4 analogue displaying native extracellular segments. In murine caecal injection experiments, co-administration of CLN4sol significantly attenuated BFT-mediated epithelial cell shedding, submucosal oedema and E-cadherin degradation compared with a nonbinding control analogue. These are acute experiments in an anatomically constrained system and do not model chronic ETBF colonisation driving progressive neoplastic change. Nevertheless, the argument for selectively targeting the receptor-binding interface rather than the BFT catalytic site is persuasive. Claudin-4 blockade would neutralise a bacterially deployed metalloprotease with no physiological substrate role in the healthy host, leaving endogenous ADAM biology intact.

This therapeutic logic is substantially de-risked by the precedent of targeting other members of the claudin family. Zolbetuximab, an antibody against the related family member claudin-18.2, was approved in 2024 for claudin-18.2-positive gastric and gastroesophageal junction cancer after demonstrating a survival benefit in two Phase III trials^[17,18]. The fact that a claudin family member is now a first-in-class approved oncology target strengthens confidence that claudin-4 itself is druggable. It also sets a cautionary expectation. Zolbetuximab's principal adverse effects are gastrointestinal, arising because claudin-18.2 retains physiological function in normal gastric mucosa even though it is more accessible on transformed or toxin-engaged cells. Claudin-4 performs an essential sealing function in tight junctions across the gut, kidney and lung, so any anti-claudin-4 strategy directed at BFT should anticipate, and design around, a similar tolerability ceiling.

The broader clinical implications extend beyond ETBF biology. ETBF colonisation is associated with early colorectal neoplasia^[2,3] and, following lamina propria niche acquisition^[19], represents a persistent oncogenic stimulus that is amenable to early intervention. A claudin-4-based neutralisation strategy could be positioned within a microbiome-informed chemoprevention framework for ETBF-colonised individuals at an elevated CRC risk, a group that may be prospectively identifiable through stool-based microbiome profiling integrated with existing CRC surveillance programmes. The observation that BFT activates Notch signalling in breast cancer cells^[20] further suggests that the claudin-4-dependent BFT substrate repertoire extends beyond colonic E-cadherin, with potential relevance to ETBF's role in breast tumorigenesis and metastatic progression, a question now directly addressable in claudin-4-competent versus claudin-4-deficient systems.

Table 1 synthesises the current evidence, principal knowledge gaps and translational priorities across each of these areas, from receptor biology to therapeutic translation and precision prevention.

Future challenges and directions

Although the identification of claudin-4 as the receptor for BFT represents a major conceptual advance, several important biological and translational questions remain. First, the current

Table 1. From claudin-4 receptors' discovery to therapeutic translation: current evidence, knowledge gaps and future priorities.

Area	Current evidence	Knowledge gap	Translational/future opportunity
BFT receptor biology	Claudin-4 is the primary epithelial receptor for BFT; claudin-3 provides partial functional redundancy	The structural basis of native BFT/claudin-4 recognition remains unresolved	Structural definition of the interaction could guide selective inhibitors
E-cadherin cleavage	Claudin-4 positions BFT to access a membrane-proximal E-cadherin cleavage site	The full repertoire of BFT substrates remains unclear	Define additional claudin-4-dependent epithelial substrates
Epithelial barrier injury	BFT-mediated E-cadherin cleavage promotes epithelial shedding and barrier disruption	The consequences of chronic/repeated toxin exposure remain poorly defined	Long-term organoid and chronic colonisation models
Inflammation and oncogenic signalling	ETBF/BFT is associated with Th17 inflammation, Wnt/ β -catenin activation and epithelial proliferation	The contribution of the claudin-4/BFT axis to tumour initiation versus progression is unresolved	Genetically engineered and tumour-prone <i>in vivo</i> models
Claudin-4 in CRC	Claudin-4 expression is dysregulated in colorectal cancer	Whether tumour-associated changes in claudin-4 alter susceptibility to BFT is unknown	Patient-derived tissues/organoids and molecular stratification
Therapeutic blockade	Soluble CLN4sol reduces BFT-mediated epithelial injury in an acute murine model	Long-term efficacy, selectivity and safety have not been established	Interface-selective antibodies, peptides or soluble receptor mimetics
Safety	Claudin-4 has important physiological epithelial barrier functions	Systemic blockade could disrupt normal tight junction biology	Selective blockade of BFT binding without interfering with physiological claudin-4
Precision prevention	ETBF is associated with colorectal neoplasia and represents a potentially identifiable microbial risk factor	Appropriate patient populations and predictive biomarkers remain undefined	Integrate stool ETBF/BFT profiling with tissue biomarkers and CRC surveillance

BFT, *B. fragilis* toxin; CLN4sol, soluble claudin-4 analogue; CRC, colorectal cancer; ETBF, enterotoxigenic *B. fragilis*.

evidence is derived largely from acute *in vitro* systems and short-term animal models, whereas ETBF-associated colorectal carcinogenesis is likely to be driven by chronic bacterial colonisation, sustained epithelial injury, persistent inflammation, immune remodelling and progressive tumour evolution. Future studies using long-term colonisation models, genetically engineered tumour-prone mice and patient-derived systems will be essential to determine how the claudin-4/BFT axis contributes to tumours' initiation and progression over time. Longitudinal studies will also help clarify whether ETBF acts as a true tumour initiator or primarily accelerates the disease in a susceptible host^[21].

Second, the biological role of claudin-4 deserves further attention. Claudin-4 is not simply a passive docking receptor; it appears to act as a spatial organiser that positions BFT at the membrane to enable E-cadherin cleavage. This raises the broader possibility that bacterial metalloproteases may exploit the host's junctional proteins to achieve substrate selectivity.

Third, therapeutic targeting requires caution. Claudin-4 has important physiological roles in epithelial barrier function, including in the gut, kidney and lung. Any claudin-4-directed intervention would therefore need to selectively block toxin engagement without disrupting normal tight junctions' biology.

Finally, the concept of microbiome-directed chemoprevention is compelling but remains to be clinically defined. Future studies should establish which patient populations are most likely to benefit, including individuals with persistent ETBF colonisation, inflammatory bowel disease, hereditary colorectal cancer syndromes or other high-risk cohorts. Integration of stool-based microbiome profiling with tissue biomarkers, including claudin-4 expression and molecular signatures of BFT activity, may enable patient stratification and precision prevention strategies. Such approaches could ultimately transform ETBF from a mechanistic biomarker of colorectal carcinogenesis into a clinically actionable target for early intervention^[22,23].

Conclusion

The identification of claudin-4 as the BFT receptor resolves a three-decade gap in bacterial pathogenesis, defines a mechanistically grounded therapeutic target and illuminates a new principle by which members of the tumour microbiota exploit the host's epithelial architecture. By establishing that receptor engagement directs a microbial metalloprotease to a membrane-proximal substrate *via* a strategy that converges with mammalian shedase biology, White et al. placed BFT in an unexpectedly sophisticated mechanistic class and, with the claudin-18.2 precedent as a guide, provide the field with one of the strongest examples to date of how a microbiome–host interaction may be therapeutically targeted. The immediate priorities are the structural resolution of the native claudin-4-BFT complex and testing of claudin-4-blocking agents in physiologically relevant models of chronic ETBF colonisation. In a field that has long been richer in epidemiological association than in actionable molecular targets, this study marks a genuine translational inflection point.

Ethical statements

Not applicable.

Author contributions

The authors confirm contributions to the work as follows: manuscript conception and writing: Monaghan TM; figure

preparation: Hatziapostolou M; manuscript revision: Polyarchou C, Hatziapostolou M, Nateri AS. All authors reviewed and approved the final version of the manuscript.

Data availability

Data sharing is not applicable to this commentary as no datasets were generated or analyzed.

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Conflict of interest

The authors have no conflict of interest to declare.

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References

- [1] Wu S, Rhee KJ, Albesiano E, Rabizadeh S, Wu X, et al. 2009. A human colonic commensal promotes colon tumorigenesis *via* activation of T helper type 17 T cell responses. *Nature Medicine* 15:1016–1022
- [2] Boleij A, Hechenbleikner EM, Goodwin AC, Badani R, Stein EM, et al. 2015. The *Bacteroides fragilis* toxin gene is prevalent in the colon mucosa of colorectal cancer patients. *Clinical Infectious Diseases* 60:208–215
- [3] Purcell RV, Pearson J, Aitchison A, Dixon L, Frizelle FA, et al. 2017. Colonization with enterotoxigenic *Bacteroides fragilis* is associated with early-stage colorectal neoplasia. *PLoS One* 12:e0171602
- [4] Wu S, Lim KC, Huang J, Saidi RF, Sears CL. 1998. *Bacteroides fragilis* enterotoxin cleaves the zonula adherens protein, E-cadherin. *Proceedings of the National Academy of Sciences of the United States of America* 95:14979–14984
- [5] Chung L, Thiele Orberg E, Geis AL, Chan JL, Fu K, et al. 2018. *Bacteroides fragilis* toxin coordinates a pro-carcinogenic inflammatory cascade *via* targeting of colonic epithelial cells. *Cell Host & Microbe* 23: 203–214.e5
- [6] Sears CL. 2009. Enterotoxigenic *Bacteroides fragilis*: a rogue among symbiotes. *Clinical Microbiology Reviews* 22:349–369
- [7] Wu S, Shin J, Zhang G, Cohen M, Franco A, et al. 2006. The *Bacteroides fragilis* toxin binds to a specific intestinal epithelial cell receptor. *Infection and Immunity* 74:5382–5390
- [8] White MT, Wang K, Zhang H, Eckhard U, Hullahalli K, et al. 2026. A pro-carcinogenic bacterial toxin binds claudin-4 to cleave E-cadherin. *Nature* 654:504–512
- [9] Luo J, Yang Q, Zhang X, Zhang Y, Wan L, et al. 2022. TFPI is a colonic crypt receptor for TcdB from hypervirulent clade 2 *C. difficile*. *Cell* 185:980–994.e15
- [10] Tao L, Zhang J, Meraner P, Tovaglieri A, Wu X, et al. 2016. Frizzled proteins are colonic epithelial receptors for *C. difficile* toxin B. *Nature* 538:350–355
- [11] Shrestha A, Uzal FA, McClane BA. 2016. The interaction of *Clostridium perfringens* enterotoxin with receptor claudins. *Anaerobe* 41:18–26
- [12] Vecchio AJ, Rathnayake SS, Stroud RM. 2021. Structural basis for *Clostridium perfringens* enterotoxin targeting of claudins at tight junctions in mammalian gut. *Proceedings of the National Academy of Sciences of the United States of America* 118:e2024651118
- [13] Cox KE, Liu S, Hoffman RM, Batra SK, Dhawan P, et al. 2024. The expression of the claudin family of proteins in colorectal cancer. *Biomolecules* 14:272
- [14] Goulas T, Arolas JL, Gomis-Rüth FX. 2011. Structure, function and latency regulation of a bacterial enterotoxin potentially derived from a

- mammalian adamalysin/ADAM xenolog. *Proceedings of the National Academy of Sciences of the United States of America* 108:1856–1861
- [15] Abramson J, Adler J, Dunger J, Evans R, Green T, et al. 2024. Accurate structure prediction of biomolecular interactions with AlphaFold 3. *Nature* 630:493–500
- [16] Lipper CH, Egan ED, Gabriel KH, Blacklow SC. 2023. Structural basis for membrane-proximal proteolysis of substrates by ADAM10. *Cell* 186:3632–3641.e10
- [17] Shah MA, Shitara K, Ajani JA, Bang YJ, Enzinger P, et al. 2023. Zolbetuximab plus CAPOX in CLDN18.2-positive gastric or gastroesophageal junction adenocarcinoma: the randomized, phase 3 GLOW trial. *Nature Medicine* 29: 2133–2141
- [18] Gorwitz GL, DeRemer DL. 2025. Zolbetuximab-clzb: targeting claudin 18.2 in advanced gastric and gastroesophageal adenocarcinoma. *Annals of Pharmacotherapy* 59:1119–1127
- [19] Hill CA, Casterline BW, Valguarnera E, Hecht AL, Shepherd ES, et al. 2024. *Bacteroides fragilis* toxin expression enables lamina propria niche acquisition in the developing mouse gut. *Nature Microbiology* 9:85–94
- [20] Parida S, Wu S, Siddharth S, Wang G, Muniraj N, et al. 2021. A procarcinogenic colon microbe promotes breast tumorigenesis and metastatic progression and concomitantly activates Notch and β -catenin axes. *Cancer Discovery* 11:1138–1157
- [21] Scott N, Whittle E, Jeraldo P, Chia N. 2022. A systemic review of the role of enterotoxigenic *Bacteroides fragilis* in colorectal cancer. *Neoplasia* 29:100797
- [22] Haghi F, Goli E, Mirzaei B, Zeighami H. 2019. The association between fecal enterotoxigenic *B. fragilis* with colorectal cancer. *BMC Cancer* 19:879
- [23] Xia S, Ma L, Li H, Li Y, Yu L. 2025. Prevalence of enterotoxigenic *Bacteroides fragilis* in patients with colorectal cancer: a systematic review and meta-analysis. *Frontiers in Cellular and Infection Microbiology* 15:1525609



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