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Dates: Received: 22 May, 2017; Accepted: 02 June, 2017; Published: 06 June, 2017

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Keywords: Biomarkers; *Drosophila*; Human; Model host; Colorectal cancer; Inflammation

<https://www.peertechz.com>

Review Article

Flies to Humans - Humans to Flies: A Virtuous Circle of Colorectal Cancer Prevention

Abstract

The two Nobel prizes in physiology or medicine of 1995 and 2011 establish *Drosophila* genetics as a significant contributor of genes and signaling pathways relevant to human disease, including innate immunity and cancer. Other than providing clues on mammalian gene homologue function, relatively little attention has been paid on the translational aspect of *Drosophila* genes, microbes and environmental factors that influence homeostasis and disease. This is particularly important for colorectal cancer (CRC) prevention, for which molecular diagnostic tools are non-existent. While clinical studies provide a wealth of information on genes and microbes linked to inflammatory bowel disease (IBD) and CRC, it is unknown if they can serve as biomarkers in terms of CRC prevention. We discuss the line of research showing that many biomarkers of intestinal inflammation and CRC in humans may be modeled and mechanistically tested in flies. *Vise versa*, genes and processes, such as regenerative inflammation and aging-associated DNA damage, found in flies to promote tumorigenesis may be tested as biomarkers of CRC risk in humans. Thus, modeling human intestinal inflammation and cancer in flies can provide a means to assess causality of conserved genes and microbes that can colonize the fly intestine. Moreover, successful modeling in flies enables the "treatability" of the pertinent biomarkers via dietary, probiotic and pharmacological interventions and paves the way for clinical trials of treatments that may alleviate intestinal inflammation and the risk for CRC.

Introduction

The genetic and histological suitability of flies for basic research on CRC

During the last two decades *Drosophila* has become a powerful model for exploring the links between inflammation and colorectal cancer (CRC). The fully sequenced genome, the high degree of human disease-related gene homology with flies (up to 75%), the reduced genetic redundancy, the great availability of genetic tools enabling spatial and temporal manipulation of cells, as well as, the evolutionary conservation of signaling pathways controlling vital biological processes and immunity, make *Drosophila* a suitable model host for the identification of candidate biomarkers implicated in tumor-promoting inflammation [1,2]. In addition, the small size, the low cost of maintenance and the easy delivery of orally administrated drugs facilitate whole-animal screening for molecular compounds affecting stem cell mediated carcinogenesis [3,4]. Remarkably, there are at least 60 chemical compounds originally known for their activity in human cells that demonstrably have the same molecular mechanism of action in flies [2].

The intestine is the most rapidly self-renewing tissue of the human body. Intestinal epithelium is continuously exposed to pathogens and chemicals of the lumen leading to enterocyte damage and concomitant regenerative inflammation that completely replenishes the damaged or lost cells by asymmetrically dividing intestinal stem cells (ISCs) [5-7]. ISCs reside at the bottom of the crypt of Lieberkühn along with the secretory Paneth cells. ISCs proliferate to give rise to transient cells that amplify and differentiate, while moving upwards the crypt. Differentiated cells are found at the rim of the crypts and the villi. These are absorptive enterocytes, but also secretory cells, namely, Paneth, endoendocrine or goblet cells. Between the villi and the lumen there is a goblet cell derived-mucus layer that protects cells from direct bacterial contact. The tissue is supported by stromal cells of various types and surrounded by visceral muscle. Similarly, *Drosophila* intestine is maintained by ISCs that divide giving rise to new ISCs and transient enteroblasts, which normally differentiate without further divisions into either absorptive enterocytes or enteroendocrine cells [8,9]. Although the mammalian Paneth, goblet and stromal cells are absent in flies, many of their immunity and barrier physiology functions are fulfilled by

highly endoreplicating enterocytes and the visceral muscle [1]. The fly intestine generally lacks crypts that support multiple progenitor cells and villi. These appear necessary for maximal nutrient absorption in mammals, but in flies a monolayer of epithelial cells surrounded by two layers of visceral muscle suffices for homeostasis. In addition to the mucus layer, the *Drosophila* gut lumen is surrounded by a chitin layer, the peritrophic matrix that confers extra protection against pathogens [10].

Using flies for identifying genetic and microbial biomarkers of risk for CRC

Fly immunity shares evolutionary conserved mechanisms with human innate immunity. *Drosophila* midgut responds to uracil from intestinal pathogenic bacteria by inducing the p38 mitogen-activated protein kinase (p38 MAPK) signaling pathway [11,12], which in turn activates the conserved NADPH oxidase, Duox, leading to the release of reactive oxygen species (ROS) [13-15]. The antimicrobial activity of ROS is complemented with the production of antimicrobial peptides (AMPs). Toll signaling pathway is responsible for the systemic AMP response mediated by the nuclear translocation of the NF- κ B-like transcription factor(s) Dorsal and/or Dif. The second NF- κ B-like pathway of *Drosophila*, named immune deficiency (IMD) pathway, is stimulated by peptidoglycan recognition proteins (PGRPs). IMD is induced by bacterial peptidoglycan leading to the nuclear translocation of Relish and AMPs expression both systemically and in the fly gut [16,17]. Intestinal damage and stress are also capable of stimulating particular AMP expression following secretion of the Upd3 inflammatory cytokine, an analog of the human interleukin (IL)-6, which activates the JAK/STAT signaling in both ISCs and the visceral muscle [18,19]. JAK/STAT pathway activation leads to the expression of epidermal growth factors and consequently induction of EGFR/Ras/MAPK cascade inducing ISC proliferation [20,21]. *Drosophila* stem cells are further modulated by the Target of Rapamycin (TOR), Hippo and wingless pathways [22-24]. This inflammation induced tissue regeneration process referred to as regenerative inflammation may contribute to tumor initiation and progression and is conserved between flies and mammals [6,7].

Inflammation is pivotal for host defense, but it can lead to pathogenesis when chronic and predispose for cancer. The inflammatory microenvironment facilitates tumor initiation and progression, although a direct causality has not been established for CRC. Germline mutations account as a driving force for the 10% of CRC incidence, whereas the vast majority of cases associate with somatic mutations and environmental factors, including chronic inflammation and bacterial infections [25,26]. For instance, the chronic inflammation of the gastrointestinal mucosa present in IBD patients is a key predisposing factor for developing CRC [27]. Nevertheless, so far only *Helicobacter pylori* infection has been established as a causative agent of gastrointestinal inflammation and cancer [28]. In mammals, areas with active inflammatory responses accompanied by a high rate of epithelial cell-turnover and

sustained DNA damage are sufficient to drive carcinogenesis [29]. This setting of increased predisposition to tumorigenesis is also found in the aging midgut of *Drosophila* [30]. Moreover, pathogenic bacterial infection promotes intestinal tumorigenesis in genetically predisposed adult *Drosophila* [31].

Previous studies have used fruit flies as model hosts to induce intrinsic and extrinsic oxidative damage that resembles the aging associated changes in progenitor cells [32]. Collective evidence indicates accumulation of γ H2AvD foci in ISCs, analogous to the mammalian γ H2AX, a DNA damage marker. γ H2AvD foci correlate with γ -ray-induced DNA damage in ISCs and age-related accumulation of 8-oxo-2'-deoxyguanosine. Interestingly, age and oxidative stress related DNA damage in *Drosophila* can be alleviated by the chemotherapy drug Metformin via downregulation of the insulin-like growth factor-I receptor/insulin receptor (IGF1R/IR) and the AKT [33]. As ISCs age, they acquire persistent chromatin lesions bearing double strand breaks (DSB) and thereby, initiating a continuous secretion of inflammatory cytokines. In mammals, damaged ISCs that have entered senescence preserve their capacity to secrete different factors and interact with the surrounding microenvironment [34]. Similarly, age-related DNA damage and JNK-driven dysplasia correlate with barrier failure and excessive systemic inflammatory signaling attributed to the bacterial translocation across the *Drosophila* gut [35,36]. Somatic inactivation of Notch tumor suppressor during aging in flies causes spontaneous neoplasia driven by somatic recombination, genomic deletions and rearrangements [30].

As microbial intestinal load increases with age, it becomes challenging to maintain symbiosis between the host and its microbes. Alterations within the microbiome structure could elicit an acute inflammatory signaling through the increased production of ROS and AMPs and the release of inflammatory cytokines and growth factors that regenerate the intestinal epithelium. However, chronic inflammatory responses and the excessive exposure of cells to oxidative stress have adverse effects on homeostasis, leading to dysbiosis. Dysbiosis is correlated with IBD [37] and cancer [38]. *Drosophila* is characterized by a simple microbiome of less than 30 microbial species [35] compared to that of humans, which is composed with hundreds of different bacterial species [39]. *Lactobacillus* and occasionally *Enterobacteriaceae* are part of both the fly and human microbiome. While huge differences exist, symbiotic bacteria are critical for host physiology in both species and the number of human bacteria that can colonize flies is far more than those found naturally. They promote growth by modulating nutrient metabolism and absorption [40] and participate in the shaping of gastrointestinal immune landscape [41,42]. Therefore, pinpointing the mechanisms by which gut microbiota affects health and disease, may help to suggest new therapeutic approaches to alleviate microbiota-directed inflammation and CRC incidence. Bacterial mono-associations or poly-associations with germ-free flies would provide insights regarding the contribution of symbiotic bacteria at the species level in intestinal disease.

Mammalian genetic and microbial biomarkers of risk for CRC

Most CRC cases are sporadic, that is, with no known genetic component attributed to them (70%–80% of all cases) and usually appear at an old age [43,44]. Hereditary forms of CRC include familial adenomatous polyposis (~1%), non-polyposis hereditary CRC or Lynch syndrome (2%–5%) and MYH-gene associated polyposis (<1%) [45]. Interestingly, molecular and cellular alterations precede morphological changes of the intestinal mucosa, and may predispose for tumorigenesis [46,47]. This must be true also for intestinal microbes and their balance [48]. Such alterations may be blamed for the recurrence of adenomatous polyps after surgical excision [49]. Therefore, ongoing efforts turn towards finding specific genetic and microbial markers that will allow the early detection of CRC appearance in terms of personalized medicine and treatment.

A hallmark of transition from normal colonic epithelium to neoplastic is genomic instability (GI), which is divided to chromosomal instability (CIN), microsatellite instability (MSI) and epigenetic instability (EI) [44]. The most familiar form of GI is CIN, which is implicated in 80%–85% of colorectal tumors [50]. GI is characterized by a) the presence of aneuploidy, which involves changes in chromosome number, b) modifications in the gene structure, such as insertions, deletions or base substitutions, which are also caused by MSI, c) chromosome rearrangements and d) gene multiplying. The basic concept of GI involves the loss of function of tumor-suppressor genes, such as *adenomatous polyposis coli* (APC), *p53*, *SMAD4*, and tumor-suppressor genes on chromosome 18q the area deleted in colon cancer (DCC), or the activation of the *K-Ras* oncogene [51].

APC is mutated in up to 80% of sporadic CRC cases and is involved in the negative regulation of Wntless-Int (WNT) signaling pathway. WNT pathway regulates various biological processes, such as cell proliferation, differentiation, polarity, and movement, and maintains intestinal epithelial cell (IEC) homeostasis [52–54]. The canonical WNT pathway is highly conserved and initiates with the binding of Wnts to Frizzled (Fz) receptors [55–57]. Downstream of Fz, Dishevelled (Dsh) the glycogen synthase kinase 3 beta (GSK3 β) and casein kinase I- γ (CK1 γ) result in the docking of the scaffold protein Axin and APC and the stabilization of β -catenin. The complex with β -catenin is disrupted upon ligand signaling and β -catenin moves to the nucleus where it binds to T-cell factor/lymphoid enhancer factor (TCF/LEF) family of transcription factors activating specific Wnt target genes. In the absence of Wnt, the serine/threonine kinases, CK1 and GSK3 α/β , phosphorylate β -catenin, which is guided by the F box/WD repeat protein β -TrCP, for degradation to the proteasome. In the absence of signaling, TCF/LEF is not activated by β -catenin and its targets are suppressed by Groucho [58]. The TCF binding sites are also similar between vertebrates and *Drosophila* [59]. The function of β -catenin as a TCF activator is strictly regulated by the multiprotein complex that involves APC, which when mutated β -catenin is activated to induce tumor-promoting genes, such as *Myc* [60].

The *KRAS* oncogene is an activated form of the endogenous gene and present in up to 43% of human CRC tumors. It encodes the guanosine diphosphate (GDP) and guanosine triphosphate (GTP) binding proteins. Wild type *KRAS* is induced by the epidermal growth factor receptor (EGFR) pathway, but the *KRAS* oncogene is constitutively active independently of such stimulation [61]. Activation of EGFR promotes an excessive mitogenic signaling cascade through the activation of numerous pathways, including the RAS – RAF – mitogen-activated protein kinase (MAPK), the phosphatidylinositol 3-kinase (PI3K) – Akt, and the phospholipase C pathway [62, 63]. *BRAF V600E* (involved in 10–15% of CRC tumors), which encodes a guanosine triphosphate (GTPase), is also involved in the EGFR pathway activation [64]. *PIK3CA* gene of PI3K pathway is mutated in ~15% of CRCs [65], while some cases include mutations in *PTEN*, a tumor suppressor, which normally inhibits PI3K [66]. Additional mutations present in CRC tumors include *FBXW7*, *TCF7L2*, *NRAS*, *FAM123B*, *CTNNB1* and *SMAD2* [67] (Table 1). Mutations in the MSI pathway are primarily due to the loss of function of DNA repair proteins, *MLH1*, *MLH3*, *PMS1*, *PMS2*, *MSH2*, *MSH3*, *MSH6* and *Exo1* [68]. MSI also affects cell mitosis (*TGF- β* , *GRB1*, *TCF-4*, *WISP3*, *activin receptor-2*, *IGF-2 receptor*, *axin-2*, and *CDX*), apoptosis (*BAX*, *caspase-5*, *RIZ*, *BCL-10*, *PTEN*, *hG4-1*, and *FAS*), and additional DNA repair genes (*MBD-4*, *BLM*, *CHK1* and *RAD50*) [69, 70]. The most known, clinically evaluated MSI markers are mononucleotide (BAT-25, BAT-26, NR-21, NR-24 and MONO-27) exhibiting high sensitivity and specificity [64] (Table 2). Two kinds of EI have been mostly described in CRC: CpG island methylator phenotype (CIMP), and global DNA hypomethylation. Both mechanisms cause silencing of gene expression. Known biomarkers for CIMP-positive tumors are *CACNA1G*, *IGF2*, *NEUROG1*, *RUNX3* and *SOCS1* and methylation must occur in at least 3 of them [71]. Indicative lists of mouse and fly homologs of human genetic and epigenetic biomarkers of inflammation and CRC are provided in Tables 1, 2.

Genetic biomarkers linked to inflammation (IBD) may also be linked to CRC. Chronic inflammation and tissue damage induce cell proliferation and aberrant differentiation of macroscopically normal-appearing colonic mucosa, which may lead to crypt enlargement and potentially to cancer initiation and progression [72–74]. The most used marker for cell proliferation during inflammation and cancer is Ki67 [75–80]. Additional markers used to estimate colorectal tumor cell mitosis are MCM7 and its negative regulator Geminin, which are involved in the DNA replication [81–83], as well as, Aurora kinase A (AURKA), which plays a critical role in cell cycle regulation [84], and proliferating cell nuclear antigen (PCNA), which is necessary for DNA synthesis during replication [85,86]. Inflammation responses involve the recruitment of tissue-resident macrophages and mast cells, which produce a variety of inflammatory mediators, including cytokines, chemokines, proteases, matrix metalloproteinases, TNF- α , interleukins (IL), interferons (IFN), and enzymes such as cyclooxygenase-2 (COX-2), 5-lipoxygenase (5LOX), and phospholipase A2 (PLA2) responsible for eicosanoid formation [87]. Many other cytokines may be pro-tumorigenic, including IL-4, IL-6, IL-8, IL-11, IL-17A, IL-22, IL-23, IL-33, TNF, TGF- β , and VEGF

Table 1: Genomic Instability and Epigenetic Instability related genes and their homology in human, mice and flies.

PROCESS	HUMAN SNPs/GENES [136]	MOUSE SNPs/GENES [137]	FLY GENES	HOMOLOGY
Chromosomal Instability	APC	APC	APC-like [138]	Human-mouse: 90%
	TP53	Trp53	p53 [139]	Human-mouse: 77%
	SMAD4	Smad4	Med [140]	Human-mouse: 98% Human-fly: 78%
	KRAS	Kras	Ras85D [141]	Human-mouse: 96% Human-fly: 85%
	MYC	Myc	dMyc [142]	Human-mouse: 87%
	RAF1	Raf1	D-raf [143]	Human-mouse: 98%
	PIK3CA	Pik3ca	PI3K [144]	Human-mouse: 99%
	BRAF	Braf	Raf [145]	Human-mouse: 86% Human-fly: 45%
	PTEN	Pten	Pten [146]	Human-mouse: 99% Human-fly: 47%
	FBXW7	Fbxw7	Archipelago [147]	Human-mouse: 95%
	TCF7L2	Tcf7l2	dTCF [59]	Human-mouse: 97%
	NRAS	Nras	NRas [148]	Human-mouse: 99%
	AMER1	Amer1		Human-mouse: 77%
	CTNNB1	Ctnnb1	arm [149]	Human-mouse: 99% Human-fly: 67%
SMAD2	Smad2	Smad2 [150]	Human-mouse: 99%	
Microsatellite Instability	MLH1	Mlh1	Mlh1 [151]	Human-mouse: 88% Human-fly: 58%
	MLH3	Mlh3		Human-mouse: 70%
	PMS1	Pms1		Human-mouse: 75%
	PMS2	Pms2	Pms2 [152]	Human-mouse: 77%
	MSH2	Msh2	spell [153]	Human-mouse: 93% Human-fly: 45%
	MSH3	Msh3		Human-mouse: 82%
	MSH6	Msh6	Msh6 [152]	Human-mouse: 85% Human-fly: 44%
	Exo1	Exo1		Human-mouse: 73%
	TGFB1	Tgfb1	Dlk1/Pref-1 [154]	Human-mouse: 90%
	PIK3R1	Pik3r1	Pi3K21B [155]	Human-mouse: 96%
	TCF-4	Tcf4	dTCF [59]	Human-mouse: 96%
	WISP3	Wisp3	Ccn [156]	Human-mouse: 79%
	ACVR2A	Acvr2a	put [157]	Human-mouse: 99% Human-fly: 47%
	IGF2R	Igf2r	DlnR [158]	Human-mouse: 81%
	AXIN2	Axin2	Daxin [159]	Human-mouse: 88%
	CDX	Cdx1		Human-mouse: 84%
	BAX	Bax		Human-mouse: 92%
	PRDM2	Prdm2		Human-mouse: 81%
	BCL10	Bcl10		Human-mouse: 91%
	PA2G4	Pa2g4	CG10576 [160]	Human-mouse: 99% Human-fly: 56%
	FAS	Fas		Human-mouse: 49%
	MBD4	Mbd4		Human-mouse: 96%
	BLM	Blm		Human-mouse: 75%
	CHEK1	Chek1	grp [161]	Human-mouse: 93% Human-fly: 47%
	RAD50	Rad50	rad50 [162]	Human-mouse: 92% Human-fly: 29%
Epigenomic Instability	CACNA1G	Cacna1g	Ca- α 1T [163]	Human-mouse: 94%
	IGF2	Igf2	Igf [164]	Human-mouse: 82%
	NEUROG1	Neurog1	Atonal [165]	Human-mouse: 77%
	RUNX3	Runx3	Runt [166]	Human-mouse: 89%
	SOCS1	Socs1	SOCS36E [167]	Human-mouse: 92%

[88]. These mediators could serve as prognostic biomarkers for CRC appearance. Additional processes and genes affecting susceptibility to intestinal infection, stress or inflammation in human, mice and flies are described in table 2 [89].

Bacteria have been linked both positively and negatively to

inflammation (IBD) and CRC. Major disruption of the healthy microbiota caused by extensive and prolonged antibiotic use, especially in neonates and children, can result in life-threatening necrotizing enterocolitis. In this case intestinal dysbiosis results in excessive inflammation, mucosal injury and cell death without regeneration [90]. Shifting the balance of intestinal

Table 2: Processes and genes affecting susceptibility to intestinal infection, stress or inflammation in humans, mice and flies, per Vaiserman, 2015 [89].

PROCESS	HUMAN SNPs/GENES [136]	MOUSE SNPs/GENES [137]	FLY GENES	HOMOLOGY
Autophagy	ATG16L1	Atg16l1	CG31033 [168]	Human-mouse: 94% Human-fly: 38%
	IRGM1	IRGM1		Human-mouse: 57%
	ULK1	ATG16L1	Atg1 [169]	Human-mouse: 89%
	MTMR3	Mtmr3	CG3632 (Myotubularin-like) [170]	Human-mouse: 84%
	VAMP3	Vamp3	n-Syb, Syb/dVAMP [171]	Human-mouse: 94%
	DAP	Dap		Human-mouse: 96%
	LRRK2	Lrrk2	dLRRK [172]	Human-mouse: 87%
	CUL2	Cul2	Cul-2 [173]	Human-mouse: 97% Human-fly: 51%
	PARK7	Park7	dj-1beta [174]	Human-mouse: 92% Human-fly: 56%
Innate Immunity	NOD2	Nod2	PGRPs [6]	Human-mouse: 79%
	RIPK3	RIPK3	Imd homolog of RIPK2 [6]	Human-mouse: 59%
	Nf-KB	Nf-KB	NF-kB-like transcription factor(s) Dorsal and/or Dif [6]	Human-mouse: 86%
	TNFa	Tnfa	Eiger [6]	Human-mouse: 79%
	COX-2	Ptgs2	COX-like [6]	Human-mouse: 87%
	TLR1-10	Tlr1-11	toll [53]	Human-mouse: 74%
	TLR-4	Tlr4	toll [53]	Human-mouse: 67%
Adaptive Immunity	IL18RAP	Il18rap		Human-mouse: 67%
	B-defensins	B-defensins	defensins [175]	
	JAK 2,3	Jak 2,3	hop [176]	Human-mouse: 94% Human-mouse: 83%
	TYK2	Tyk2	Tyk2 [177]	Human-mouse: 78%
	IL-1	Il1		Human-mouse: 98% Human-mouse: 68%
	SLC11A1	Slc11a1	Nramp [178]	Human-mouse: 89%
	FCGR2A	Fcgr3		Human-mouse: 60%
	FCGR2B	Fcgr2b		Human-mouse: 56%
	REL	Rel	Rel [179]	Human-mouse: 70%
	CARD9	Card9		Human-mouse: 86%
	MIF	Mif		Human-mouse: 90%
	FOXO3	Foxo3	dFOXO [180]	Human-mouse: 94%
	PRDM1	Prdm1		Human-mouse: 87%
LSP1	Lsp1		Human-mouse: 68%	
SMAD3	Smad3	Smox [181]	Human-mouse: 100% Human-fly: 81%	
SMAD7	Smad7	MAD [182]	Human-mouse: 98%	
TGF-β	Tgfb1		Human-mouse: 90%	
TNFRSF6	Fas		Human-mouse: 49%	
TNFRSF9	Tnfrsf9		Human-mouse: 57%	
TNFRSF14	Tnfrsf14		Human-mouse: 46%	
IL4	Il4		Human-mouse: 41%	
IL6	Il6	Upd [6]	Human-mouse: 40%	
IL10	Il10		Human-mouse: 73%	
IL12A	Il12a		Human-mouse: 59%	
IL12B	Il12b		Human-mouse: 66%	
IL13	Il13		Human-mouse: 58%	
IL17A	Il17a		Human-mouse: 62%	
IL18	Il18		Human-mouse: 65%	
IL22	Il22		Human-mouse: 78%	
IL1R	Il1r		Human-mouse: 69%	
IL7R	Il7r		Human-mouse: 63%	
IL8R	Cxcr2		Human-mouse: 71%	
IL17AR	Il17ar		Human-mouse: 70%	
IL23R	Il23r		Human-mouse: 67%	
IFNG	Ifng		Human-mouse: 41%	
VNN1	Vnn1		Human-mouse: 76%	



	TNFSF8	Tnfsf8		Human-mouse: 70%
	TNFSF11	Tnfsf11		Human-mouse: 84%
	TNFSF15	Tnfsf15		Human-mouse: 65%
	CCR3	Ccr3		Human-mouse: 70%
	CCR9	Ccr9		Human-mouse: 86%
	CXCR3	Cxcr3		Human-mouse: 86%
	CXCR4	Cxcr4		Human-mouse: 89%
	CXCL1	Cxcl1		Human-mouse: 73%
	IL5	Il5		Human-mouse: 72%
	GATA3	Gata3	Pannier [183]	Human-mouse: 96%
	DENND1B	Dennd1b		Human-mouse: 83%
	LNPEP	Lnpep		Human-mouse: 88%
Regeneration	Wnt	Wnt	Wnt [184]	
	Notch	Notch	Notch [185]	Human-mouse: 90% Human-fly: 49%
	APC	APC	APC-like [138]	Human-mouse: 90%
	STAT3	Stat3	Jak/Stat [19]	Human-mouse: 99%
	STAT4	Stat4	Jak/Stat [19]	Human-mouse: 95%
	STAT5	Stat5	Jak/Stat [19]	Human-mouse: 96%
	JAK2	JAK2	Jak/Stat [19]	Human-mouse: 94%
	KRAS	Kras	Ras85D [141]	Human-mouse: 96% Human-fly: 85%
	PIK3CA	Pik3ca	PI3K [144]	Human-mouse: 99%
Oxidative stress	NOS2	Nos2	dNOS [186]	Human-mouse: 81%
	DUOX2	Duox2	Duox [187]	Human-mouse: 84% Human-fly: 38%
	ADO	Ado	CG7550 [188]	Human-mouse: 86% Human-fly: 31%
	SLC22A4	Slc22a4	Orct [189]	Human-mouse: 85%
	GPX1/4	Gpx1/4	GTPx-1 [190]	Human-mouse: 93%
	UTS2	Uts2		Human-mouse: 50%
	PEX13	Pex13	Pex13 [188]	Human-mouse: 91% Human-fly: 36%
	PARK7	Park7	DJ-1alpha [191]	Human-mouse: 92% Human-fly: 53%
	DLD	Dld	DI [192]	Human-mouse: 88% Human-fly: 49%
	BACH2	Bach2		Human-mouse: 89%
	LRRK2	Lrrk2		Human-mouse: 87%
	PRDX5	Prdx5	Prx5 [188]	Human-mouse: 93% Human-fly: 57%
	CARD9	Card9		Human-mouse: 86%
	SLC23A1	Slc23a1		Human-mouse: 89%
		NOX1	Nox1	Nox [193]
Epithelial Barrier	MUC1	MUC1	Mucin-like [194]	
	MUC3	A630081 J09Rik	Mucin-like [194]	Human-mouse: 64%
	MUC4	MUC4	Mucin-like [194]	
	MUC5B	Muc5b	Mucin-like [194]	Human-mouse: 76%
	HNF4A	Hnf4a	HNF4a [195]	Human-mouse: 96%
	CDH1	Cdh1	Cdh1 [196]	Human-mouse: 81%
	CDH2	Cdh2	CadN2 [197]	Human-mouse: 97%
	CDH3	Cdh3		Human-mouse: 82%
	LAMB1	Lamb1	LanB1 [198]	Human-mouse: 93%
	GNA12	Gna12	Cta [199]	Human-mouse: 94%
	CDH11	Cdh11	Cadherin domain CG11059 [200]	Human-mouse: 97%
	ERRFI1	Errf1		Human-mouse: 82%
	ITLN1	Itln1		Human-mouse: 81%
	MMP1	Mmp1	dm1- and dm2-MMPs [201]	Human-mouse: 59%
	MMP3	Mmp3	dm1- and dm2-MMPs [201]	Human-mouse: 77%
	MMP7	Mmp7	dm1- and dm2-MMPs [201]	Human-mouse: 70%
	MMP9	Mmp9	dm1- and dm2-MMPs [201]	Human-mouse: 81%
	MMP10	Mmp10	dm1- and dm2-MMPs [201]	Human-mouse: 76%

	MMP12	Mmp12	dm1- and dm2-MMPs [201]	Human-mouse: 62%
	MMP13	Mmp13	dm1- and dm2-MMPs [201]	Human-mouse: 86%
	MMP14	Mmp14	Mmp1 [202]	Human-mouse: 97% Human-fly: 39%
	TIMP1	Timp1		Human-mouse: 74%
	TIMP2	Timp2		Human-mouse: 98%
	TIMP3	Timp3	dN-TIMP [201]	Human-mouse: 96%
	DLG5	Dlg5	Dlg5 [203]	Human-mouse: 92%
	MLCK	Mylk3	Strn-Mlck [204]	Human-mouse: 68%
Additional Pro-tumorigenic	IL11	Il11		Human-mouse: 88%
	IL23	Il23		Human-mouse: 74%
	IL33	Il33		Human-mouse: 52%
	VEGF	Vegf	Pvf1/3 - VEGF-related factor 1/3 [66]	Human-mouse: 88%

microbiota from a pathogenic to a protective complement of bacteria can protect the gut from inflammation and subsequent injury [91]. Accordingly, broad-spectrum antibiotics destroy the flora leading to intestinal inflammation and damage that can be prevented with oral administration of microbiota-derived molecular patterns, such as lipopolysaccharide (LPS) that induce a steady state of inflammatory cytokines and prime the epithelium again pathogens [92]. From the immunological perspective evidence reveals various properties of intestinal bacteria that distinguish them as commensals vs. pathogens [93]. Nevertheless, for the most part microbiota is just linked to the healthy or the diseased state with only a few clear cases of bacterial pathogens presented as causal for the disease. Reduced abundance of potentially beneficial bacteria has been reported for patients with IBD and CRC (Table 3). Some of these belong to the family *Lachnospiraceae*, which produce short chain fatty acids, such as the anti-inflammatory butyric acids [94] and include the genera *Lachnospira* [95–97], *Blautia* [95,98–102], *Anaerostipes* [95] and *Roseburia* [103–106], even though some reports indicate increase abundance in CRC [107,108]. The genus *Lactobacillus* was negatively correlated with CRC [109–115]. *Lactobacillus* strains have been well characterized for their probiotic properties including production of butyrate, which has an antiinflammatory function [94], as well as, the antibacterial lactic acid and bacteriocins. In addition, Lactobacilli reduce the secretion of virulence factors from enterovirulent pathogens alleviating their deleterious effects on the host [116]. Similarly, Clostridia members of clusters XIVa, IV and XVIII, have been reported to reduce inflammation [117] and have decreased abundance in IBD [117–122]. Moreover, some reports have found reduced amounts of *Bifidobacteria* in CRC patients [95], while others the opposite [123].

Translational studies for identifying “treatable” biomarkers of risk for CRC using *Drosophila*

Deciphering the right inflammatory status in the intestine is necessary for designing clinical trials against IBD and maybe CRC. Mucosal healing in IBD patients has shown promise as it correlates with remission of ulcers in Crohn’s disease and erosions and ulcers in ulcerative colitis [7,124]. Transcriptomic studies in humans have led to the identification of genetic

and microbial associations with IBD and CRC. Here, we emphasize the use of *Drosophila* as a whole-animal model to validate the effectiveness, causality and toxicity of identified “treatable” biomarkers in intestinal disease. Examples include the “humanized” *Drosophila* strains, which are genetically engineered to express human orthologs [2,125]. Notably, tens of chemicals originally selected to target human proteins, such as Rapamycin, BEZ235, SP600125 and DAPT, have been shown to have the same mechanism of action in *Drosophila* i.e. inhibition of PI3K/mTOR, JNK and γ -secretase/Notch signaling, respectively [125–130]. Thus, accumulating evidence suggests that *Drosophila* could fill the gap between *in vitro* and mammalian model host testing (Figure 1).

Moreover, flies could be used to examine the association between identified intestinal disease-related microbiota and host. For instance, the commensal microbe of *Drosophila* *Acetobacter pomorum* was found to modulate insulin pathway via acetic acid production and subsequently promote ISC proliferation and overall animal growth [131]. Also, a positive correlation between *Enterococcus* spp. and IBD patients has been also reported. *Enterococcus* strains that form better biofilms, adhere strongly on intestinal cells and possess antioxidant defense mechanisms are mostly found in IBD patients versus healthy people [132]. Therefore, dissecting host-microbiome interactions of overrepresented in IBD and CRC bacterial strains, such as *Bacteroides*, *Escherichia*, *Enterococcus* and *Enterobacter* in gnotobiotic flies could give insights regarding bacterial pathogenicity. This is more feasible nowadays due to the increasing number of human microbiota species that we are able to culture and thus test in model organisms, such as flies [133]. Similarly, *Drosophila* could be used to determine the beneficial impact of bacteria underrepresented in CRC like *Clostridia* and *Lactobacillus*.

Accumulated evidence in *Drosophila* highlights the role of diet in intestinal disease. Nutrient deprivation and reduced insulin pathway correlate with reduced ISC proliferation and number, a phenotype that is reversible upon feeding [134]. Dietary L-glutamate also stimulates intestinal cell proliferation and growth via regulation of Ca²⁺ signaling [135]. This plasticity of ISC to nutrient availability could be used to target

Table 3: Genera over/under represented in IBD and CRC and their oxygen sensitivity.

GENERA	OXYGEN SENSITIVITY	REPRESENTATION IN IBD AND/OR CRC	CULTIVATED
Bacteroides	strictly anaerobic	Overrepresented in CRC	[133]
Escherichia	facultatively anaerobic	Overrepresented in CRC	
Enterococcus	facultatively anaerobic	Overrepresented in CRC	
Enterobacter	facultatively anaerobic	Overrepresented in CRC	
Prevotella	strictly anaerobic	Overrepresented in CRC	
Ruminococcus	strictly anaerobic	Overrepresented in IBD/ Underrepresented in CRC	
Porphyromonas	strictly anaerobic	Overrepresented in CRC	
Streptococcus	facultatively anaerobic	Overrepresented in CRC	
Peptostreptococcus	facultatively anaerobic	Overrepresented in CRC	
Fusobacterium	strictly anaerobic	Overrepresented in CRC	
Erysipelothrix	facultatively anaerobic	Overrepresented in IBD and CRC/ Underrepresented in IBD	[205]
Bifidobacterium	strictly anaerobic	Overrepresented/ Underrepresented in IBD and CRC	[133]
Lachnospira	strictly anaerobic	Underrepresented in IBD and CRC	
Blautia	strictly anaerobic	Underrepresented in IBD and CRC	
Anaerostipes	strictly anaerobic	Underrepresented in IBD and CRC	
Roseburia	strictly anaerobic	Overrepresented in CRC/ Underrepresented in IBD and CRC	
Lactobacillus	facultatively anaerobic	Underrepresented in IBD and CRC	
Clostridia members of clusters XIVa, IV and XVIII	strictly anaerobes	Underrepresented in IBD	

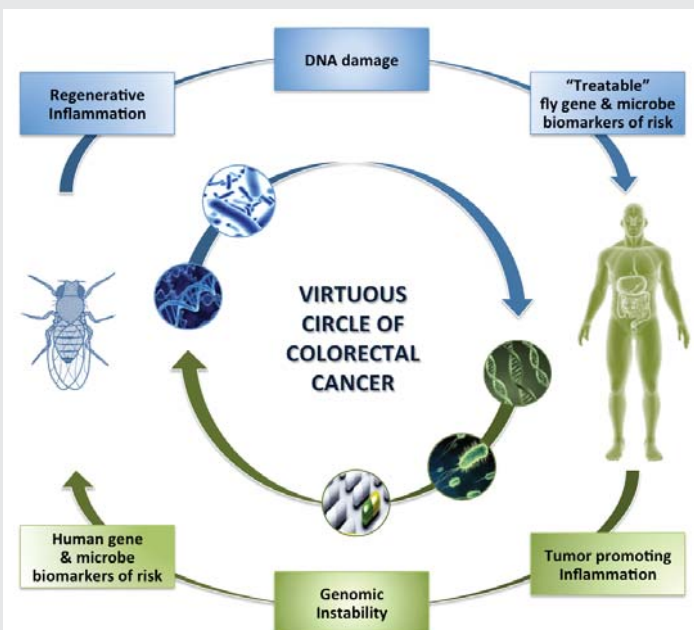


Figure 1: A virtuous circle of research on CRC prevention. Work on the facilitating hallmarks of cancer, tumor-promoting inflammation and genomic instability, can in addition to unbiased approaches pinpoint genetic and microbial biomarkers linked to risk for CRC. Biomarkers can be further tested and manipulated therapeutically by diet, drug and probiotic administration in flies to assess causality and facilitate clinical trials in humans.

the aberrant proliferation of dysplastic lesions. Given that CRC is a multifactorial disease, a sophisticated combination of probiotic, chemical and dietary interventions might be required to efficiently prevent the disease. In this regard, tumor-initiating inflammation may be successfully targeted by sequestration of regenerative chemokines/cytokines and selective inhibition of signaling molecules that promote tumor survival and growth [25].

Limitations

The use of animal models provides the ability to study the effects of biomarkers of fundamental signaling pathways, microbes and environmental factors and suggest therapeutic interventions against intestinal inflammation and tumorigenesis. A practical limitation of using *Drosophila* in translational studies on IBD and CRC is the inability to assess the disease promoting properties of human anaerobes that are highly sensitive to the presence of oxygen. An additional limitation of the fly model is the lack of adaptive immunity and the absence of lamina propria in which immune cells reside and infiltrate. Thus, alternative animal hosts such as mouse models should be used to validate and complement the assessment of biomarkers, especially those related to adaptive immunity and highly sensitive to oxygen microbes. Regardless, advantages such as the short lifespan of the fly facilitate assessments of drug-diet-microbial interventions against sporadic intestinal cancer during ageing that is impractical to perform in mice. Thus, *Drosophila* can be an attractive model host for studying well-conserved genetic, microbial, and environmental components of intestinal homeostasis and disease, the analogous features of which might play a pivotal role in human health.

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