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1 **Mutagenesis by microbe: The role of the microbiota in shaping the**
2 **cancer genome**

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14 **Keywords**

15 microbiota; microbiome; DNA damage; mutational mechanism; mutational signatures

16 **Abstract**

17 Cancers arise through the process of somatic evolution fuelled by the inception of somatic
18 mutations. We lack a complete understanding of the sources of these somatic mutations.
19 Humans host a vast repertoire of microbes collectively known as the microbiota. The
20 microbiota plays a role in altering the tumour microenvironment and proliferation. In
21 addition, microbes have been shown to elicit DNA damage which provides the substrate for
22 somatic mutations. An understanding of microbiota-driven mutational mechanism would
23 contribute to a more complete understanding of the origins of the cancer genome. Here
24 we review the modes by which microbes stimulate DNA damage and the effect of these
25 phenomena upon the cancer genomic architecture, specifically in the form of mutational
26 spectra and mutational signatures.

27

28 **Origin of the cancer genome and the role of the microbiota**

29 Oncogenesis is driven by the Darwinian selection of somatic mutations (see Glossary) over
30 time [1]. Mutations arise through the formation of genetic aberrations and their subsequent
31 interactions with the DNA repair machinery and cell cycle related pathways including DNA
32 synthesis[2]. Mutational mechanisms alter the DNA in distinguishing manners resulting in
33 genetic patterns known as mutational signatures (Box 1).

34 The origin of mutations allows them to be classified into three categories, which are (i)
35 inherited genetic variants that lead to an increase in the risk of cancer development. (ii)
36 Environmental factors, exogenous factors including UV light, tobacco smoking and diet that
37 mutate the DNA and that are directly linked to cancer. (ii) Stochastic errors associated with
38 DNA replication and other phenomena. These are seemingly inevitable random mutations
39 which arise due to the intrinsic properties of DNA biology. Seminal work by Tomasetti and
40 Vogelstein showed that about two-thirds of the mutations in the cancer genome originate
41 from stochastic events [3, 4].

42 Lung and cervical adenocarcinoma genomes harbour median values of 33% and 83%
43 stochastic mutations respectively [3]. However, epidemiologic evidence indicates that a high
44 proportion (~90%) of cases are attributable to environmental factors, i.e. tobacco smoking
45 and HPV infection, respectively. The managing of environmental risk factors is thus crucial in
46 cancer prevention, even though stochastic/replicative mechanisms are the major drivers
47 (See ref 3 for a more detailed discussion). However a complete catalogue of environmental
48 factors that contribute cancer risk is lacking. A large number of known carcinogens promote
49 oncogenesis by causing mutagenesis e.g. ultraviolet light, ethanol, tobacco smoke and
50 radioactive substances.

51 The human microbiota is increasingly recognized as an emerging environmental risk factor.
52 The human microbiota is home to about 3.8×10^{13} bacterial cells and it is estimated that the
53 collective metagenome of these bacteria encompasses about 100 times more genes than
54 the human genome [5, 6]. Although the majority of studies focus on bacteria, upon which
55 this review is focussed, the human microbiota includes members from all 5 kingdoms of life
56 as well as viruses. A large number of studies demonstrate that microbiota features are
57 involved in the development and progression of a range of cancers. The term 'oncobiome'

58 has been coined to describe the relationship between the microbiota and cancers[7].
59 However, oncobiome research has identified relationships that are primarily correlative
60 rather than causative in nature. With regard to the putative mechanistic role that the
61 microbiota has in cancer development, immune modulation in the form of inflammation
62 caused by the microbiota is an intense area of research [8]. Effort has also been made in
63 defining the role of the microbiota in cell proliferation [9].

64 The microbiota is known to be involved in a diverse assortment of mutational mechanisms
65 (Table 1). Known variation in cancer risk due to unknown environmental factors could be
66 explained in part by variations in the ability of the microbiota of individual subjects to
67 induce DNA-damage and thus somatic mutations. Here we describe the current state of
68 knowledge on microbes and their ability to compromise the stability of the human genome
69 ultimately leading to cancer.

70 In this review we describe the microbiota influences on genome integrity through (i) direct
71 DNA damage, (ii) immune cell induced DNA damage, (iii) dietary interaction, and (iv)
72 disruption to the DNA damage response.

73

74 **Direct DNA Damage**

75 Members of the microbiota can produce proteins, molecules and secondary metabolites
76 that can directly cause DNA damage. These products can interact directly with the host
77 DNA thereby mutating it.

78

79 *Colibactin*

80 *Escherichia coli* is classified into 4 phylogenetic groups, A, B1, B2, and D. About 30–50% of
81 *E. coli* strains identified in stool microbiota of individuals from high-income nations belong
82 to group B2. Within the B2 group, 35% of isolates possess genomic islands known as *pks* (for
83 polyketide synthase) islands[10]. The 54-kb *pks* island is a biosynthetic gene cluster
84 encoding for a non-ribosomal peptide synthetase (NRPS)–polyketide synthase (PKS) hybrid
85 gene cluster, which encodes for colibactin [11]. Colibactin can cause Double-strand breaks
86 (DSB) in mammalian DNA thereby promoting genome instability and an increase in mutation

87 rate [12, 13]. It is not currently known how colibactin is transported from the cell exterior all
88 the way into the nucleus. The *pks+* *E. coli* strains are over-represented in the gut of
89 individuals with colorectal cancer, being detected at a rate of 20% in the mucosa of healthy
90 individuals but 55%-67% in patients with colorectal cancer (CRC) [14, 15]. Furthermore,
91 *pks+* *E. coli* was disproportionately frequently identified in subjects with familial
92 adenomatous polyposis (FAP) compared to healthy controls [16]. Monocolonization of
93 azoxymethane (AOM)-treated IL10^{-/-} mice with *pks+* *E. coli* promoted tumorigenesis, while
94 challenge with strains lacking *pks* reduces the frequency of tumorigenesis [14].

95 Colibactin cross-links directly with DNA through an electrophilic cyclopropane moiety
96 'warhead' [17]. Liquid chromatography-mass spectrometry-based methodologies have
97 identified that colibactin alkylation of DNA via the cyclopropane warhead results in adenine-
98 colibactin adducts [18, 19]. This phenomenon was identified in both HeLa cells and in mouse
99 models [19]. Colibactin can also induce DNA inter-strand cross-links and activation of the
100 DNA damage response including Fanconi anemia DNA repair [20]. Recent structural analysis
101 revealed that colibactin contains two conjoined warheads enabling its ability to cause DNA
102 crosslinks [21]. Double strands breaks are not believed to be a direct consequence of
103 colibactin activity but rather occur due to replication stress caused by DNA cross-links [20].
104 Recent sequencing analysis of colibactin-induced DSB sites revealed that these DSBs
105 occurred at AT-rich regions and in particularly at the pentanucleotides motif containing
106 AAWWTT [22]. Single nucleotide variants at the AAWWTT were found to be enriched in a
107 number of cancers including CRC and stomach cancer compared with a WWWW motif.
108 Two mutational signatures were found to be linked with the AAWWTT colibactin motif,
109 SBS28 and SBS41[22]. Mutational signature SBS28 has been associated with POLE mutation
110 while Mutational signature SBS41 has no known aetiology.

111

112 *Cytolethal distending toxin (CDT)*

113 The cytolethal distending toxin (CDT) is produced by an array of gram-negative bacteria
114 within the gamma and epsilon classes of the phylum Proteobacteria[23]. It is a heat-labile
115 exotoxin whose properties lead it to be classified as a both a cyclomodulin and a genotoxin.

116 The proteobacteria that can produce CDT are sub-dominant members of the human gut
117 microbiota.

118 CDT is a heteromultimeric protein comprised of three subunits, CdtA, CdtB and CdtC
119 which are encoded within a bacterial single operon [24, 25]. Subunits CdtA and CdtC
120 function to allow delivery and internalization of CDT into target cells[25]. CdtB shares
121 sequence, structural and functional homology with DNase I and is highly conserved among
122 bacteria [26, 27]. Furthermore, nuclear localization signals have been identified in CdtB
123 proteins [28]. Studies with ApcMin/+ mice that are genetically susceptible to small bowel
124 cancer found that a *Campylobacter jejuni* strain harbouring the CDT operon promoted
125 colorectal tumorigenesis compared to treatment with non-CDT bacterial controls, while
126 mutation of the cdtB subunit attenuated this phenomenon [29]. CdtB has been shown to
127 promote DSB *in vitro* and *in vivo* [26, 30, 31]. However, the current model of CdtB activity
128 holds that CdtB acts in a dose-dependent manner and tends not to induce double strand
129 breaks directly [32]. At low to moderate doses, CdtB causes single strand breaks (SSB) which
130 are addressed by Single-strand break repair (SSBR)[33]. If CDT-induced SSBs are not
131 addressed before replication or occur during replication, they may cause a stalled
132 replication fork [32, 33]. At high doses, CDT can induce DSB directly by two cuts to the DNA
133 backbone that are juxtaposed to each other [32].

134

135 *Reactive oxygen species*

136 Reactive oxygen species (ROS) are a chemically reactive family of molecules containing
137 oxygen which include the highly reactive hydroxyl radical (OH⁻), superoxide radical (O₂⁻),
138 and non-radical hydrogen peroxide (H₂O₂). Reactions of ROS with DNA generates oxidative
139 DNA base lesions. To date, more than 30 oxidative DNA base lesions have been
140 identified(Box 2)[34].

141 Microbiota activity is known to produce reactive oxygen species through varied means. For
142 example, primary bile acids, cholic acid (CA) and chenodeoxycholic acid; (CDCA) are
143 synthesised by the liver and are secreted into the small intestine from the gall bladder. A
144 small proportion of these bile salts are transformed into secondary bile salts by the gut
145 microbiota. These secondary bile salts are thought to be involved in the production of ROS

146 [35]. The production of secondary bile in the colon where the bacterial metabolic repertoire
147 exist maybe be one of the reasons that CRC is more prevalent than small intestine cancer
148 although differences in stem cell turnover is likely a more important factor[3].

149 Hydrogen sulphide (H₂S) is produced by the metabolic activity of colonic bacteria including
150 taurine desulfonation by *Bilophila wadsworthia*, cysteine degradation by *Fusobacterium*
151 *nucleatum* and sulfonate degradation by sulfate-reducing bacterium such as *Desulfovibrio*
152 *desulfuricans*. Increased relative abundance of such bacteria has been linked to CRC
153 development [36, 37]. Evidence suggests that H₂S production leads to DNA damage partly
154 due to ROS generation [37, 38].

155 *Dinitrogen trioxide and nitrosative deamination*

156 Nitrosative deamination is deamination mediated by dinitrogen trioxide (N₂O₃, nitrous
157 anhydride). In this phenomenon, dinitrogen trioxide can react with nucleotides and induce
158 deamination by nucleophilic aromatic substitution. These events are mutagenic because the
159 resulting deaminated bases may be read incorrectly if not repaired[39].

160 Dinitrogen trioxide can be generated from the autooxidation of nitric oxide (NO-) or the
161 condensation of nitrous acid (HNO₂)[40]. GIT microbes can produce endogenous nitric oxide
162 and/or nitrous acid by four mechanisms: (i) The hemethiolate monooxygenase, nitric oxide
163 synthase (NOS), oxidises L-arginine (Arg) to produce nitric oxide [41] (ii) Denitrification of
164 nitrate (NO₃⁻) to nitrogen (N₂), which is an important part of the nitrogen cycle and is carried
165 out by denitrifying bacteria and plants. During denitrification, nitric oxide is produced by
166 one-electron reduction of nitrite (NO₂⁻) by heme or Cu-containing nitrite reductases[42]. (iii)
167 Respiratory nitrite ammonification (also referred to as dissimilatory nitrate reduction to
168 ammonium)[42]. (iv) Acidic non-enzymatic reduction of nitrite to NO which is driven by
169 lactic acid bacteria such as lactobacilli and bifidobacteria[43].

170

171 **Immune cell induced DNA damage**

172 The microbiota and immune system closely interact from the early stages of human
173 development. In this section we review mechanisms by which the microbiota can influence
174 immune cells to behave in a genotoxic manner.

175

176

177 *Hypochlorous acid (HOCl) production*

178 Neutrophils, which are a type of polymorphonuclear leukocyte, accumulate at sites of injury
179 with the primary function of promoting inflammation. Neutrophils produce a potent
180 antimicrobial known as hypochlorous acid (HOCl) which is produced by myeloperoxidase
181 using as substrates the chloride ions and hydrogen peroxide (H₂O₂) produced by NADPH
182 oxidase [44]. HOCl is highly reactive and readily interacts with DNA. HOCl has been shown to
183 cause a cytosine to 5-chlorocytosine (5ClC) conversion [45]. This in turn can cause a C to T
184 transition during replication.

185 In addition, HOCl can induce the peroxidation of lipids leading to the formation of
186 malondialdehyde (MDA). Studies in both cellular and animal models found that such a
187 production of MDA can lead to a significant increase in the formation of 3-(2-deoxy-β-D-
188 erythro-pentofuranosyl)pyrimido[1,2-α]purin-10(3H)-one (M1dG) , a damaged guanine.
189 [46]. M1dG adducts are mutagenic causing G>T and G >A substitutions.[47]

190 The microbiota is now known to be a modulator of neutrophilic biology[48]. A recent study
191 in a mouse model demonstrated that neutrophil pro-inflammatory activity correlates
192 positively with neutrophil ageing while in circulation[49]. Furthermore the study found that
193 the microbiota regulates neutrophil ageing by Toll-like receptor and myeloid differentiation
194 factor 88-mediated signalling pathways[49]. A depletion of the microbiota was mirrored in
195 the number of aged neutrophils and an improvement in inflammatory disease.

196

197 *Hypobromous acid production*

198 Eosinophils are granular leukocytes with a multifunctional role in immune biology.
199 Eosinophils secrete eosinophil peroxidase which catalyzes the formation of hypobromous
200 acid (HOBO) from hydrogen peroxide and halide ions (Br⁻) in solution. HOBO can also be
201 produced by reaction of HOCl with Br⁻ ions. Like HOCl, HOBO is an oxidant and functions to
202 oxidize the cellular components of invading pathogens; however excess production of HOBO
203 can also lead to host damage including DNA damage, namely the formation of 8-bromo-2'-

204 deoxyguanosine and 5-bromo-2'-deoxycytidine. A SupF forward mutation assay in human
205 cells found that the prominent mutation induced was G >T mutation but HOBO also induces
206 G>C, G>A, and delG [50].

207

208 *Activation-induced cytidine deaminase*

209 Activation-induced cytidine deaminase (AID) is a member of the cytidine deaminase family
210 of enzymes with a role in somatic hypermutation. Immunohistochemistry identified the
211 ectopic overexpression of AID in inflamed tissue derived from patients with Crohn's disease
212 and ulcerative colitis as well as colitis-associated colorectal cancers [51]. The expression of
213 AID in colonic epithelial cell lines induced an increase in the mutation rates in these cells
214 [51]. Knock-out of AID in IL10 null mice attenuated the mutation rate in their colonic cells
215 and also inhibits CRC development[52]. Inflammation seems to be key to this aberrant
216 activity. *H. pylori* infection, which is known to induce inflammation, promotes ectopic
217 expression of AID in non-tumorous epithelial tissues [53]

218 Whole genome analyses in chronic lymphocytic leukaemia revealed that the activity of AID
219 may produces two types of substitution pattern (i) a 'canonical AID signature' characterised
220 by C to T/G substitutions at WRCY motifs near active transcriptional start sites and (ii) a
221 'non-canonical AID signature' characterised by A to C mutations at WA (W=A or T) motifs
222 occurring genome-wide in a non-clustered fashion [54]. These mutational processes have
223 been assigned to mutational signatures SBS84 and SBS85[55].

224

225 *By-stander effect and Enterococcus faecalis*

226 *Enterococcus faecalis* is known to promote CRC oncogenesis in interleukin 10 -/- mice [56].
227 *E. faecalis* can promote the bystander effect which leads to double-stranded DNA breaks,
228 tetraploidy and chromosomal instability. In this model, *E. faecalis* production of
229 extracellular superoxide induces polarization of macrophages to an M1 phenotype [57-59].
230 In turn macrophages produce 4-hydroxy-2-nonenal (4-HNE), a diffusible breakdown product
231 of ω -6 polyunsaturated fatty acids whose expression in this context is dependent on

232 Cyclooxygenase-2[60, 61]. Primary murine colon epithelial cells exposed to polarized
233 macrophages or purified 4-HNE undergo transformation [62].

234 **Dietary interaction**

235 The diet of the host and the gut microbiota are inextricably linked. GIT bacteria depend
236 almost exclusively on the host diet for their nutritional substrates (a restricted number of
237 taxa can metabolize mucins and glycoproteins) and indeed the composition of the
238 microbiome is correlated strongly with diet. Diet is a key modulator of cancer risk. In the
239 cases described below, microbiota-diet interactions lead to the formation of genotoxic
240 compounds capable of mutating the host genome.

241

242 *N-nitroso compounds (NOCs)*

243 NOCs, such as nitrosamines and nitrosamide, are known to be potent carcinogens. NOCs
244 are formed by the nitrosation of secondary amines and amides via nitrosating agents, such
245 as N_2O_3 and N_2O_4 [63]. NOCs can be found in foods such as processed meats, smoked/cured
246 fish and German beer[64]. Additional compounds such as nitrate and nitrite which are
247 precursors to nitrosating agents can be found in food including vegetables which may
248 account for 50–70% of an individual's intake of nitrate and nitrite [65]. Endogenous NOCs
249 are also formed and in many cases, this is because of the activities of microbes. Firstly,
250 bacteria produce nitrosating agents (See Dinitrogen trioxide and nitrosative deamination).
251 Further amines and amides are produced by bacterial decarboxylation of amino acids [65].
252 Heme has been suggested to catalyse the formation of NOCs[66]. Inhibitors of nitrosation
253 are ingested as part of a diet and include vitamin C, vitamin E and polyphenols[67].

254 The activated form of NOCs induce a number of methylated DNA adducts (of which over 12
255 are known) by SN_1 -nucleophilic substitution[68]. These alkylated DNA bases can be
256 mutagenic if not repaired before replication[69]. SBS mutational signature 11 has been
257 linked to the mutagenic activity of alkylating agents [70].

258

259 *Acetaldehyde*

260 Alcohol is classified as a Group 1 carcinogen (carcinogenic to humans). Worldwide, 3.6% of
261 all cancer deaths and 3.5% of all cancer cases are attributable to alcohol consumption[71].
262 Ethanol (C₂H₅OH), the psychoactive ingredient in alcoholic beverages, is believed to be the
263 major causative compound of cancer in alcoholic beverages.

264 Ethanol is introduced into a catabolic pathway where it is broken down and the metabolites
265 expelled via the urinary system. Ethanol is first metabolized by alcohol dehydrogenase
266 (ADH), cytochrome P4502E1 (CYP2E1) and catalase thereby forming acetaldehyde (ethanal).
267 Acetaldehyde is further oxidised by aldehyde dehydrogenase to produce acetate.
268 Aldehydes cause DNA damage in the form of double strand breaks and the Fanconi anaemia
269 pathway is responsible for the repair of this damage [72]. Aldehydes has been
270 demonstrated to cause intrastrand crosslink between adjacent guanine bases[73]. This can
271 lead to the mutagenic event of GG>TT double base substitution which is a characteristics of
272 Mutational signature DBS2 [55, 73].

273 Bacteria can not only produce ethanol but also break it down into acetaldehyde. Oral taxa
274 are known to be able to produce acetaldehyde from ethanol or glucose [74]. In addition, gut
275 microbes can also produce acetaldehyde from sugars [75]. Indeed there have been reports
276 of bacterial autobrewery syndrome (intoxication by ethanol formed by fermentation by
277 microbes in the gut) in which a strain of *Klebsiella pneumoniae* was implicated [76]. This
278 strain was also strongly associated with non-alcoholic fatty liver disease and fatty liver
279 disease symptoms in a mouse model. Mutational signature 16 has been link to alcohol
280 consumption [77].

281

282 **Disruption to the DNA damage response**

283 Human DNA experiences repeated events of DNA damage throughout the cell cycle. The cell
284 has a complex network of systems whose purpose is to ensure the fidelity of DNA. Known as
285 the DNA damage response, this cellular system is responsible for detecting DNA damage,
286 signalling its presence, promoting DNA repair cell cycle checkpoint and/or apoptosis.

287 The mismatch repair mechanism is responsible for addressing base-base mismatches and
288 insertion/deletion mis-pairs generated during DNA replication and recombination[78].

289 Enteropathogenic *Escherichia coli* was found to promote the depletion of MSH2 and MLH1
290 proteins, which are crucially important for mismatch repair in cell models[79]. This
291 phenomenon was found to be dependent on the bacterial type-3 secretion effector
292 EspF[79]. Furthermore, mitochondrial targeting of EspF was necessary for this activity.
293 Colonic epithelial cells infected with Enteropathogenic *E. coli* display an increased mutation
294 rate particularly in microsatellite DNA sequences.

295 The human gastric pathogen *Helicobacter pylori* also inhibits the expression of MMR gene
296 expression, in part through the modulation of miRNAs [80, 81].

297

298 Mutational signature 6 is characterised by C>T transitions at an NpCpG trinucleotide context
299 [82]. This mutational signature is associated with small indels (usually 1-3bp) at nucleotide
300 repeats. This indel pattern is equivalent to phenomena known as microsatellite instability.
301 Microsatellite instability is caused by aberrations in the DNA mismatch repair (MMR)
302 machinery. The origin of MMR deficiencies is genetic and/or epigenetic alterations in MMR
303 genes. Microsatellite instability occurs in 15% of CRC genomes; 3% are associated with
304 Lynch syndrome while 12% are associated with sporadic CRC [83].

305

306 **Mutational signatures as a tool to study the effect of microbes on the human** 307 **genome**

308 Multiomic experimental designs are supremely placed to delineate the relationship between
309 the microbiota and the architecture of the cancer genome. Population studies in which
310 both cancer genomic and microbiome are assessed can provide information on the
311 interaction between the cancer genetic architecture and the microbiota. However, there is a
312 fundamental caveat with this type of experimental design. Cancer can take many years to
313 form, and mutational mechanisms act at different time points of the natural tumour history.
314 Furthermore, composition of the microbiota at most body sites is usually dynamic. Thus, a
315 single snap shot of the microbiota may not be wholly related to the mutational signatures
316 then identified. A prospective study where individual's microbiota are determined at pre-
317 and post-transformation stages would allow for more informative comparisons between the

318 microbiota and pre-transformation mutational mechanisms. Additionally, individuals with
319 pre-cancer lesions such as Barrett's oesophagus may be prime candidates to study due to
320 their increased propensity to develop cancer. Studying cancer heterogeneity and
321 evolutionary dynamics could allow for the identification of the timing of mutational
322 mechanisms. Furthermore, recent advancements have allowed for mutational signature
323 extraction from non-cancerous tissue thus allowing elucidation of microbial associated
324 mechanisms prior to transformation [84]. Experiments in which a microbe or a community
325 of microbes are grown in the context of a model such as a cell line or organoids would help
326 to eliminate confounders and make more direct correlations. Dziubańska-Kusibab and
327 colleagues used cultured cell lines exposed to colibactin to identify its DNA sequence
328 targets. Furthermore this target sequence was then cross-referenced with mutational
329 signatures derived in population cancer genomic data to find clinically associated mutational
330 signatures (See colibactin section).

331

332

333

334

335 **Concluding Remarks**

336 Cancer prevention is relatively under-researched when compared to therapeutic
337 development, with only 2 to 9% of funding put towards this area [85]. A high proportion of
338 cancer cases and cancer deaths could be avoided through modification of environmental
339 risk factors. About 42% of cancer incidences in the US are estimated as being attributable to
340 modifiable risk factors - this figure is also reflected in the UK population [86]. Evidence is
341 building in favour of the microbiota as an environmental modulator of cancer risk. We
342 outlined the multitude of ways that the metabolic activities of members of the human
343 microbiota can lead to mutations.

344 Our ability to modulate the microbiota is improving steadily, featuring diet, antibiotics,
345 phage therapy, faecal microbiota transplantation (FMT), prebiotics, probiotics and Live
346 Biotherapeutics[87]. Thus one could plausibly develop strategies to alter the structure of an

347 individual's microbiota in order to reduce its mutagenic potential (see Outstanding
348 Questions).

349 In order to make informed decisions on therapeutic interventions, a complete catalogue of
350 microbial-associated mutational mechanisms is required. Furthermore, the relative impact
351 of each mutational mechanisms on the cancer genome need to be delineated. Microbial-
352 associated mutational mechanisms which have both been found in a wide range of cancers
353 as well as contributing to a great number of mutations will take priority when deciding what
354 mechanisms need to be addressed first.

355 We propose to leverage advancements in cancer genomics, namely in the form of
356 mutational signatures, to associate microbes to mutational mechanisms. These can provide
357 qualitative and quantitative information on the mutagenic effect that microbes undoubtedly
358 have.

359 It is possible that certain aspects of the microbiota activity protect against mutagenesis and
360 cancer. These potential mechanism need to be elucidated to enable the harnessing the
361 microbiota as prophylactic agents.

362

363

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368

369 **Glossary**

370 **Base substitutions:** A type of mutation in which one base is replaced by another in DNA.

371 **Chromosomal instability:** A phenomena which leads to alterations in chromosome number
372 and/or structure.

373 **DNA adduct:** Formed by the addition of a chemical moiety to a DNA base

374 **DNA alkylation:** The addition of an alkyl group (C_nH_{2n+1}) to a DNA base

375 **DNA crosslinking:** Formation of covalent bonds between two nucleotides. This bond can be
376 formed between nucleotides on the same DNA stand (intrastrand crosslinks) or different
377 strands (interstrand crosslinks)

378 **DNA deamination:** The removal of an amino group from a DNA base.

379 **DNA repair:** A diverse collection of pathways with the purpose of addressing DNA damage
380 and maintaining genome stability.

381 **Double-strand breaks:** This is where both strands of DNA which are juxtaposed to each
382 other

383 **Environmental risk factor:** A thing or process which is not inherited that increases the risk
384 for a particular disease.

385 **Microbes:** Microorganisms including bacteria, fungi, protists and virus. Usually exist as a
386 single cell organism.

387 **Microbiome:** The combined genetic material of the microorganisms in a particular niche.

388 **Microbiota:** The collection of organisms in a niche.

389 **Mutational mechanism:** Biological phenomena which lead to the generation of mutations.
390 Usually involving DNA damage, DNA repair and DNA replication.

391 **Mutational signature:** The characteristic DNA pattern of mutations produced by a
392 mutational mechanism.

393 **Oncogenesis:** The transformation of a normal cell into a cancer cell.

394 **Oxidative Base Lesions:** DNA Bases that occur due to a reaction with Reactive oxygen
395 species

396 **Somatic mutation:** A mutation which occurs in a somatic cell and is thus not heritable.

397
398
399
400

401 **Box1 | Mutational signatures**

402 Specific mutational mechanisms produce characteristic patterns in the genome known as
403 mutational signatures. Recent advances in mathematical modelling and bioinformatics have
404 led to great improvements in our ability to identify mutational signatures from cancer
405 genomic data. There are six defined classes of base substitutions: C>A, C>G, C>T, T>A, T>C
406 and T>G [note: In accordance with the Catalogue of Somatic Mutations in Cancer (COSMIC)
407 system, all substitutions are referred to by the pyrimidine of the mutated Watson-Crick base
408 pair]. The incorporation of the 5' and 3' bases flanking the mutated base of the six originally
409 defined classes gives an expanded classification system of 96 possible mutations. Utilizing
410 this 96-class system as the framework and applying non-negative matrix factorization and
411 model selection, with input from genomic data from 7042 cancer samples from 31 different
412 cancer types, 21 mutational signatures were initially identified [82]. With the inclusion of
413 more genomes for a heterogeneity of cancers, as well as the consideration of single base
414 insertion/deletions and double base substitutions, the number of mutational signatures has
415 expanded[55]. Currently, the number and type of mutational signatures characterised are as
416 follows: 49 single base substitutions, 11 doublet base substitutions, four clustered base
417 substitutions (DBS), and 17 small insertion and deletion (indels) mutational signatures[55].
418 Structural variants also occur in cancer genomes and they include insertions, deletions,
419 inversions, balanced or unbalanced translocations, amplifications and complex
420 rearrangements on a scale of >50 bp in size[88]. Efforts have also been made to define the
421 signatures of these events [89]. Mutational signatures provide an insight into the mutational
422 mechanisms that act on a cancer genome over time. Mutational signatures are typically
423 displayed as histogram with the frequency of base substations (or indels or doublet base
424 substitutions) with respect to the genomic context. SBS signature 1 is characterised by C>T
425 transversions at methylated CpG sites within an NpCpG trinucleotide context. The putative
426 mechanisms behind SBS signature 1 is spontaneous or enzymatic deamination of 5-
427 methylcytosine to thymine. This newly formed thymine maybe base-paired with adenine
428 during replication, provided DNA repair is not executed. Many mutational signatures
429 described do not have a known aetiology.

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Table 1. Microbial associated mechanisms and genomic consequences

Source	Involvement of microbiota features	Key role in a mutational mechanism	Postulated effected on cancer genomic landscape	Reference
Activation-induced cytidine deaminase (AID)	<i>Helicobacter pylori</i> infection cause ectopic expression of AID	Cytosine deamination at specific motifs	Mutational signatures SBS84 and SBS85	[53, 55]
Acetaldehyde	Various inhabitants of produce ethanol and are capable metabolic act on it to produces acetaldehyde	N2-ethylidenedeoxyguanosine, Guanine- guanine intrastrand crosslinks	GG-to-TT base substitution. Mutational signature DBS2	[73]
Colibactin	Expressed by <i>Escherichia coli</i> containing a <i>pks</i> island	Adenine – adenine intra-strand crosslinks, Double strand breaks,	DSBs at an AAWWTT pentanucleotides motif. Mutational signatures SBS28 and SBS41	[22]
Cytolethal distending toxin (CDT)	Produced by various Gram-negative bacteria including enteropathogenic <i>Escherichia coli</i> , <i>Campylobacter</i> species, <i>Shigella</i> species and <i>Haemophilus ducreyi</i>	Single strand breaks and Double-strand breaks	Infidelity of DNA repair can lead to structural variants such as indels	[55]
Disruption of DNA mismatch repair	<i>Helicobacter pylori</i> and Enteropathogenic	Deletion of MMR proteins	Microsatellite instability, Mutational	[79, 80, 82]

	<i>Escherichia coli</i> can disrupt mismatch repair		signature SBS6, ID1 and ID2	
Dinitrogen trioxide	Metabolic activities of the microbiota can produce precursors to N ₂ O ₃ e.g. denitrifying bacteria	Nitrosative deamination	Various base substitutions e.g. Adenine nitrosative deamination to Hypoxanthine can lead to T>A substitution	[39, 42]
Hypobromous acid	Eosinophil's produce Hypobromous acid. The microbiota can influence eosinophilic biology	8-bromoguanine	G > T primarily but also G > C, G > A, and delG	[50]
Hypochlorous acid	HOCl is produced by Neutrophils. The microbiota can influence neutrophil inflammatory status	Formation of 5-chlorocytosine (5ClC), formation of malondialdehyde	C>T, G >A, G>T substitutions	[45, 46]
N-nitroso compounds (NOCs)	Microbes play a role in the production of nitrosating agents and produce biogenic amine	Alkylated DNA base	Various base substitutions e.g. O ⁶ -methylguanine (O ⁶ -MeG) can cause a G(C)>A(T) transition	[69]
Reactive oxygen species	Various metabolic activities	Oxidative Base Lesions	G to T transversion, SBS Mutational signatures 18 and 36	[90]
4-hydroxy-2-nonenal	<i>Enterococcus faecalis</i> induces the bystander effect via polarising	Exocyclic HNE-DNA adducts	Chromosomal instability	[60]

	macrophages. Polarised macrophages produces 4- hydroxy-2-nonenal			
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437 **Box 2 | Oxidative DNA Base Lesions**

438 Guanine has the lowest redox potential of the native bases and is thus the most readily
 439 oxidised. Two common oxidative base lesions which are generated by the oxidation of
 440 Guanine include 8-oxo-7,8-dihydro-2'-deoxyguanosine and 2,6-diamino-4-oxo-5-
 441 formamidopyrimidine (FapyG) which occur at an estimated rate of 1000–2000 and 1500–
 442 2500 per cell/per day in normal tissues, respectively[91]. Furthermore, the occurrence and
 443 the mutagenicity of these oxidative DNA base lesions vary considerable. For example, 7,8-
 444 dihydro-8-oxo-guanine is about four times as mutagenic and four times more frequent in its
 445 occurrence than 7,8-dihydro-8-oxo-adenine[91, 92]. Replication of DNA containing 8-oxo-
 446 7,8-dihydro-2'-deoxyguanosine and 2,6-diamino-4-oxo-5-formamidopyrimidine (FapyG) are
 447 shown to induce G:C to T:A (C >A) and G:C to T:A (C >A) respectively[93].

448 The nucleobases within the cellular nucleotide pool may also undergo oxidation.
 449 Misincorporation of these nucleoside triphosphates can induce mutations. The two major
 450 products of nucleotide pool oxidation are 8-hydroxy-2'-deoxyguanosine 5'-triphosphate (8-
 451 OH-dGTP) and 2-hydroxydeoxyadenosine 5'-triphosphate (2-OH-dATP). 8-OH-dGTP has
 452 been demonstrated to induce A:T to C:G transversions when introduced into COS-7
 453 mammalian cells[94]. *In vitro* analysis using HeLa cell extract showed that 2-OH-dATP within
 454 the nucleotide pool can led to G·C to A·T (C>T) transitions and G·C to T·A(C>A)[95].

455 Mutational signatures 18 and 36 have been suggested to be attributed to reactive oxygen
 456 species. Mutational signature 36 has been specifically attributed to ROS in the context of
 457 MUTYH-Associated Polyposis (MAP) syndrome [90]. MAP syndrome is defined by biallelic
 458 germline mutation of MUTYH gene and is a colorectal polyposis which predisposes
 459 individuals to CRC. MUTYH DNA glycosylase is coded by the MUTYH gene and functions to

460 prevent 8-Oxoguanine-related mutagenesis by scanning the newly-synthesized daughter
461 strand in order locate and remove incorporated adenine paired with 8-Oxoguanine[93].

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