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1 **Phages enter the fight against colorectal cancer**

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24 **Summary**

25 Intestinal microbiota undergo significant changes in colorectal cancer (CRC). Zheng *et al.*
26 (Nature Biomed Eng. 2019) observe detrimental overpopulation of *Fusobacterium*
27 *nucleatum* (*F. nucleatum*) in mice and patients, suppressing the beneficial butyrate-
28 producing *Clostridium butyricum*. Phage-guided irinotecan-loaded dextran nanoparticles
29 promote release of bacterial-derived butyrate, while *F. nucleatum* and CRC cells are
30 eliminated. These findings describe a possible novel therapeutic strategy for CRC.

31

32 **Keywords**

33 Bacteriophage; colorectal cancer; chemotherapy; microbiota

34

35 Colorectal cancer (CRC) is one of the three leading causes of cancer-related deaths
36 worldwide. This multi-factorial and multi-stage disease impacts the life of millions of
37 newly-diagnosed patients annually. Due to disease recurrence and metastasis, clinical
38 management of CRC patients is associated with high healthcare costs. **Currently**, novel
39 therapeutic strategies are urgently needed. A recently reported alternative approach has
40 been to treat patients with bacteriophage (phage) for different pathological conditions.
41 These bacteria-killing viruses can be classified as lytic or temperate units, as they either
42 undergo lytic or lysogenic cycles, respectively. Although both events lead to bacterial
43 destruction, lysogenic events require that viral and bacterial genomes integrate and
44 replicate during the virus's dormant phase [1-4].

45 Recently, Zheng *et al.* [1] identified that CRC patients and $Apc^{Min/+}$ mice exhibit a
46 significant increase of *Fusobacterium nucleatum* (*F. nucleatum*), which is typically found

47 in oral and nasal cavities. As an alien bacterial species to the intestines, *F. nucleatum*
48 produces immune blocking agents and competes with the beneficial butyrate-producing
49 *Clostridium butyricum* (*C. butyricum*) units, thus promoting CRC development and
50 enhancing chemotherapy resistance. In human saliva, the authors isolated a temperate
51 phage capable of specialized and targeted killing of *F. nucleatum* without impacting the
52 *C. butyricum* population. From this, the authors went on to treat piglets with phage-guided
53 dextran nanoparticles. Their experiments reveal that in response to this therapeutic
54 strategy, minimal changes occur in vital metabolic or immunological functions. They
55 further developed a phage-guided biotic-abiotic hybrid nanosystem that could increase
56 the chemotherapeutic potency of irinotecan against CRC cells whilst also selectively
57 killing the *F. nucleatum* population and allowing the butyrate-producing bacteria to expand
58 in numbers at the same time. Phages have a long history of usage with varying success
59 (**Figure 1**). Careful consideration should thus be given to this exciting discovery, as this
60 new therapeutic strategy of administering phage-guided irinotecan-loaded dextran
61 nanoparticles may impact CRC treatment in coming years.

62 It should be appreciated that the composition of the intestinal phage population
63 has been reported to be altered at different stages of CRC development. *Parabacteroides*
64 phage YZ-2015b units undergo an exponential increase from early to late CRC stages
65 [5]. In keeping with this idea, adherent invasive *Escherichia coli* promotes tumor growth
66 in *APC^{Min/+}* mice, while phage treatment has been found capable of increasing survival
67 and reducing tumor growth in these bacteria-infected mice. However, elimination of
68 cancer-causing bacteria *via* phages appears to worsen inflammatory bowel disease, as
69 they alter the intestinal immunity. Studies have found lower phage levels in responsive

70 rather than non-responsive ulcerative colitis patients who received fecal microbiota
71 transplantation since phages increase the release of interferon γ (IFN- γ) in this non-
72 responders group [2]. Another study further reveals that colitis changes the intestinal
73 phage population toward a stochastic state of dysbiosis in mice and humans [3].
74 Moreover, intestinal inflammation has been shown to promote bacterial pathogenic
75 evolution through a disease-driven transfer of temperate phages, as it supports the
76 expression of phage promoter *Tum*, free phage production and transfer, and bacterial
77 SOS response [4]. Collectively, these findings should focus our attention on the fact that
78 phage therapy may lead to unforeseeable side effects in humans.

79 Treating humans with bioengineered phages has, however, been proven a
80 promising therapeutic strategy in cases where traditional approaches have failed.
81 Successfully, Spencer and colleagues applied a three-phage cocktail in a teenager
82 patient who underwent bilateral lung transplantation but had a drug-resistant bacterial
83 infection. Following 121 days of phage treatment, this patient exhibited a favorable clinical
84 improvement, including enhanced liver function and significant resolution of infection sites
85 [6]. Another exciting investigation involved the use of engineered virus-like particles with
86 multiple IgG-binding ZZ domains from Q β phage capsids. This molecular strategy
87 enabled the specific elimination of human pluripotent stem cells by 5-fluorocytosine
88 without impacting on the non-target differentiated cellular population [7].

89 Although the idea of Zhang and colleagues to block CRC development by
90 enhancing bacteria-producing butyrate through bioengineering-related methods is indeed
91 remarkable and mechanistically attractive [1], there is a wealth of evidence countenancing
92 caution to be considered. For instance, dextran has been chemically bound to 2-

93 nitroimidazole (NI). NI can be metabolized into bioreductive species that block DNA
94 synthesis and damage several intracellular targets in both eukaryotic and prokaryotic
95 cells, leading to nonselective cell death. Compounds of this class have indeed been
96 suggested to act as radiosensitisers [8]. This illustrates the carcinogenic potential that this
97 class of compounds have been reported to possess [9]. On the other hand, Park *et al.*
98 report that Gram-positive commensal bacteria control the intestinal epithelial cell turnover
99 by releasing short-chain fatty acids [10]. Stappenbeck and colleagues then revealed that
100 while the short-chain fatty acid butyrate is essential to colonocytes' metabolism, it inhibits
101 forkhead box o3 (FOXO3) transcriptional activity impairing proliferation in the colonic stem
102 cell niche [11]. However, Merchant and colleagues indicated that in *Apc* mutant stem
103 cells, in which β -catenin signaling is high, butyrate-dependent regulation through zinc
104 finger DNA-binding protein 89 (ZBP-89) promotes the development of early CRC stages
105 [12]. These facts should stimulate a thoughtful debate on the diverse effects of butyrate
106 on stem cells in physiological or malignant colon conditions, as it could significantly impact
107 cancer relapse and the patient's overall survival.

108 We believe that the findings of Zheng *et al.* embrace a promising and feasible
109 therapeutic strategy for CRC patients, as it provides a significant advancement in
110 therapies that strengthens the body's anticancer mechanisms with chemotherapy.
111 Further development should shed light on how this type of treatment reinvigorates the
112 anti-tumor immunity and impacts the behavior of cancer stem cells in CRC cases.

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143 **Figure legend**

144 **Figure 1** – A century of phage research and therapy. Following the discovery of phages
145 at the beginning of the 20th century, this class of bacteria-killing viruses was successfully
146 applied in the treatment of a broad range of bacterial infections. However, unfortunate
147 events impaired the evolution of such therapies in Western countries for more than a half-
148 century. The urgent emerging need for treatments for extensively drug-resistant or totally
149 drug-resistant bacteria has led the scientific community to revisit the potential application
150 of phages in treating a wide range of conditions. This has been expanded to include
151 cancer.