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Phages enter the fight against colorectal cancer

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Summary

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

Keywords

Bacteriophage; colorectal cancer; chemotherapy; microbiota

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

Recently, Zheng *et al.* [1] identified that CRC patients and $Apc^{Min/+}$ mice exhibit a 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

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

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

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

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

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

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Figure legend

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