Graphical Review

Functional role of the cancer microbiome in the solid tumour niche

Erin A. Marshall [*], Nikita Telkar, Wan L. Lam

Integrative Oncology, BC Cancer Research Centre, Vancouver, BC, Canada

A B S T R A C T

The importance of gut microbiome to cancer therapy and response cannot be overstated, however the contribution of the bacterial population to the local solid tumour ecosystem is often overlooked. Seminal studies of tumour-resident microbiomes have shown that relative abundances of specific bacteria in the tumour correlate with survival metrics, implicating the microbiome in patient outcome. Similarly, patterns of microbiome community shifts between tumour-bearing and unaffected organs suggests a role for the tumour microbiome niche in contributing to tumour biology and behaviour. Recent reports of the detection of bacteria in solid tumours of diverse human organs have provided a strong rationale for deciphering the role of the solid-tumour microbiome across all human-host anatomic and physiologic niches, as the microbiome is ubiquitously present throughout the human body. Here, we review the role of the human microbiome in mediating response to therapies, as well as the differences between tumour and non-malignant-resident communities. We discuss the ability of the tumour microbiome to interact with the host, thereby influencing host cell behaviour and cancer-associated processes. Further, we evaluate recent technological advances that allow us to actively quantify these populations and the relationships between cell types. Finally, we suggest how these dynamic interactions can be harnessed for therapeutic benefit in the treatment of cancer.

1. Introduction

In the human body, bacteria outnumber humans in number of cells and number of genes expressed. The bacterial communities are highly diverse, and their composition is dependent on their niche in the body, as the availability to oxygen and nutrients varies. While well-studied in the gut, bacteria invade other barrier tissues and organs, including the lungs, which are long thought to be sterile barring infection. In cancer, understanding cell populations in the tumour microenvironment (TME) is crucial, as it represents opportunities for therapeutic intervention and prevention (Xavier, Young et al.). The gut-resident microbiome composition has a demonstrated effect on patient treatment outcomes, both in traditional chemotherapies, as well as in immunotherapy (Fig. 1A). In mouse models, a large class of gut commensals (*Gammaproteobacteria*) are able to metabolize Gemicitabine, which is a common therapeutic for pancreatic cancer, into its inactive form of 2′,2′-difluorodeoxyuridine (Geller et al.,2017). In the context of immunotherapy, the essentiality of a microbiome to treatment response has been solidified, however both the exact mechanism of action and identity of bacterial mediators remains elusive (Gopalakrishnan et al.,2018, Matson et al.,2018, Routy et al.,2018). However, the enrichment of *Bacteriodetes* in immunotherapy

responders may provide new therapeutic targets for increasing patient response rate.

2. The tumour niche is distinct and selects for bacteria that are able to live in the tumour microenvironment

The microbiome in the gut has been well characterized, and the composition of this community is directly related to cancer patient outcome (Zitvogel et al.,2018). However, the microbial niche is not just limited to the gut: they are pervasive throughout the human body (Fig. 1B). Foundational studies have shown that the tissue-resident microbiome is significantly altered in composition from that of tumour-free organs, and that the presence of a tumour in an organ acts as a selection pressure both to the bacteria that reside within the tumour, and also to the bacteria in the surrounding non-malignant tissue (Riquelme et al.,2019). Across most tumour types, a marked difference in detectable species has been observed, but this has not held true for community diversity measures, such as alpha (within tumour) and beta (non-malignant vs. tumour) diversity. Differences in diversity have only been observed when compared to non-cancerous hospital control biopsies, possibly indicative of the unique selection pressure caused by
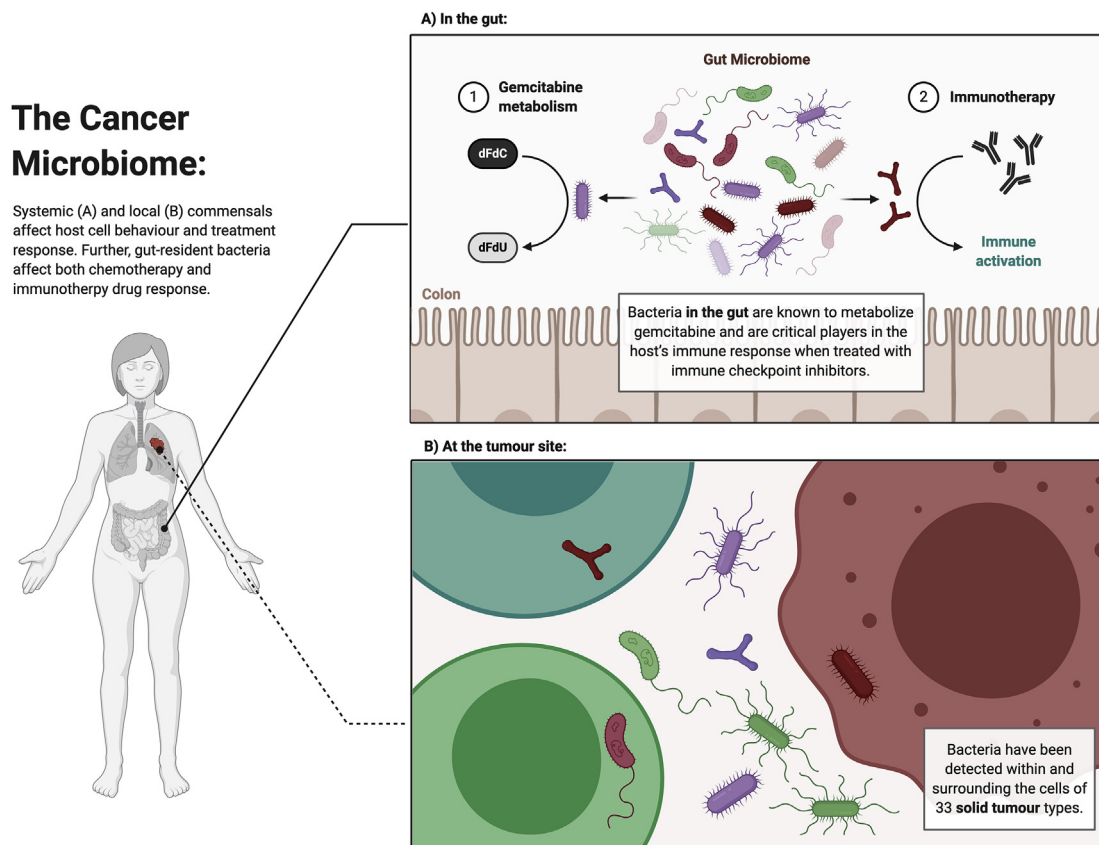

Fig. 1. Effects of microbiome on cancer. The human microbiome can affect cancer progression through roles directly at the tumour site, as well as in the gut. A) In the gut, bacterial relative abundance measures have been associated with cancer survival. Furthermore, the presence of specific bacteria in the gut has the ability to metabolize the chemotherapeutic agent gemcitabine, decreasing its effectiveness. For immunotherapy, the presence of a microbiome is necessary to mediate the effectiveness of immune checkpoint blockade drugs. B) At the cancer site, bacteria are present both in the tumour milieu, and also inside tumour cells.

tumour proximity. Further, recent microbiome models have shown that there is specific colonization of bacteria from the colon distinctly to the tumour and non-malignant region, supporting the hypothesis that the tumour and non-malignant tissues present as unique niches for bacterial inhabitancy (Fig. 2) (Riquelme et al.,2019). This is of particular interest in the context of metastasis, where the distant site is under a new selection pressure (Kostic et al.,2012), but the cancer cells traveling to the site have the capacity to bring inhabiting bacteria with them to these sites, simultaneously affecting the distant metastatic niche (Xavier et al., 2020).

3. Tissue-resident microbiome affects tumour microenvironment

At the tumour site, understanding the composition of bacteria is important, due to their ability to interact with host cells that they surround. Interkingdom signaling allows for communication between commensals, pathogens, and hosts (Hughes and Sperandio, 2008). In the TME, bacteria have the capacity to influence their hosts both directly (through receptor-mediated interactions) as well as indirectly through hormone and metabolite release (Fig. 3).

In a manner similar to host cell-cell interaction, bacterial cells in the TME interact with host cell receptors, influencing cell signaling and stimulating cancer-associate processes. In particular, bacterial receptors and ligands have been shown to stimulate G-protein coupled receptors (GPCRs) and epidermal growth factor receptor (EGFR), causing signaling through the cancer-associated MAPK, Akt, and JNK pathways (Fig. 3A) (Moghal and Sternberg, 1999, Cohen et al.,2017). Also relevant to the TME, bacteria have a well-characterized role in stimulating the human immune system, in particular, through LPS binding to TLR4 in gram-negative bacteria (Fig. 3B) (Rosadini and Kagan, 2017). As such,

this TME-receptor interaction cannot be understated.

The microbiome is also able to interact with cancer cells indirectly by influencing the TME nutrient availability and extrinsic cell factors. In particular, the presence of bacteria at the tumour site can influence the tumour milieu via the generation of ROS and subsequent MAPK/NFKB activation (Yardeni et al.,2019), as well as by affecting to and responding to changes in pH (Fig. 3C) (Ilhan et al.,2017). Further, as all other cells in the tumour microenvironment, bacteria are able to directly provide and compete with tumour cells for metabolites (protein, lipids, and amino acid derivatives) (Fig. 3D). This is significant, considering the limiting availability of essential nutrients, which cannot be synthesized by human cells and must be acquired via circulation. Further, a recent 16S rDNA FISH study at the tumour site suggested that bacteria are present within tumour cells indicating that bacteria could indeed be influencing cancer cell signaling from inside the cell by local nutrient provisioning (Nejman et al.,2020). In parallel to providing certain metabolites to the cancer cells, they need to consume nutrients to live, and are thus in direct competition with tumour cells in the nutrient-poor TME.

4. Methods of capturing microbial information from tumour tissue

Undoubtedly, to understand the role of the microbiome in the solid tumour niche requires capturing the true diversity of the patient samples. However, capturing the diversity and composition of the tumour-resident human microbiome presents with its own unique set of challenges: as these microbes are living communities, any attempt at culturing *ex vivo* or *in vivo* imparts a selection pressure on the bacterial community, skewing the population relative abundances. Consequently, most studies that quantify changes in bacterial relative abundance in human samples are

Tumour-resident microbiome is unique from non-malignant microbiome
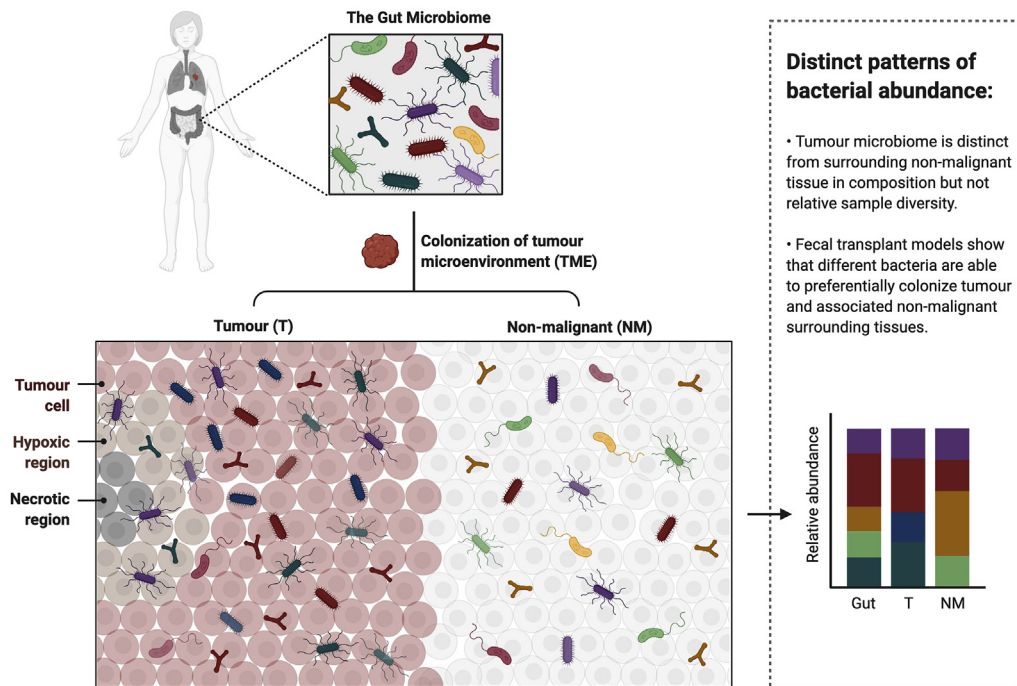

Fig. 2. Tumour-resident microbiome is specific and unique from non-malignant microbiome. The tumour-resident microbiome is distinct from the microbiome resident to the surrounding non-malignant tissues. Fecal matter transplant models have shown that distinct portions of the adopted microbiome are able to colonize these sites. While changes in the microbial composition have been shown between tumour and adjacent non-malignant tissues, metrics of bacterial diversity in solid tumours have differing assessments of population diversity. Some studies show tumours to have less diverse microbiomes than corresponding non-malignant tissues, while other studies suggest that a difference in alpha diversity is only observable when tissue from diseased and non-diseased organs is compared.
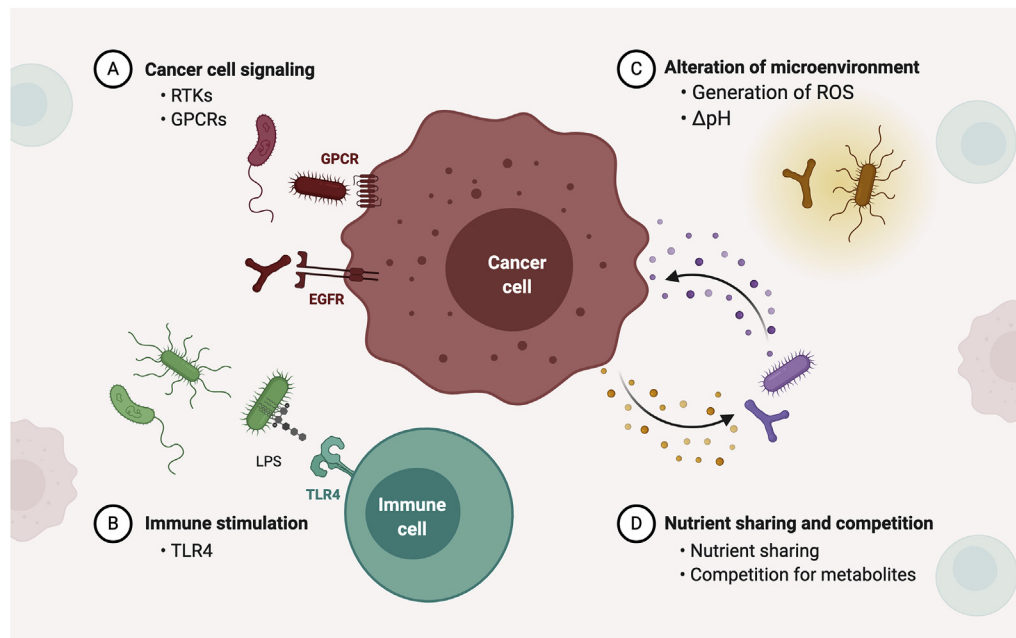

Fig. 3. Tissue-resident microbiome affects tumour microenvironment. Bacteria affect tumour behaviour through four main mechanisms: directly through stimulation of tumour and immune cells, as well as through altering the microenvironment and competition for nutrient indirectly. A) Bacteria are able to directly interact with cancer cells and initiate signaling cascade via activation of GPCRs and RTKs. B) Gram-negative bacteria stimulate the human immune system by binding to TLK4 and causing a local immune response. C) The presence of bacteria in the tumour microenvironment are able to indirectly affect tumour cell behaviour by locally altering pH and generating ROS. D) Bacteria are able to indirectly affect cancer cell metabolism through the excretion of molecules that affect host cells and through the competition for essential metabolites within the tumour.

descriptive – without culturing and experimental perturbation – correlating population changes with disease status in order to identify candidate organisms and determine their (targetable) features that may be influencing cancer behaviour.

From bulk tissues, DNA and RNA profiling are commonly accessible methods of capturing microbial information from sequencing reads – DNA profiling of the biomass or limiting to living cells via RNA transcripts (Fig. 4A). However, as bacterial sequence reads are often
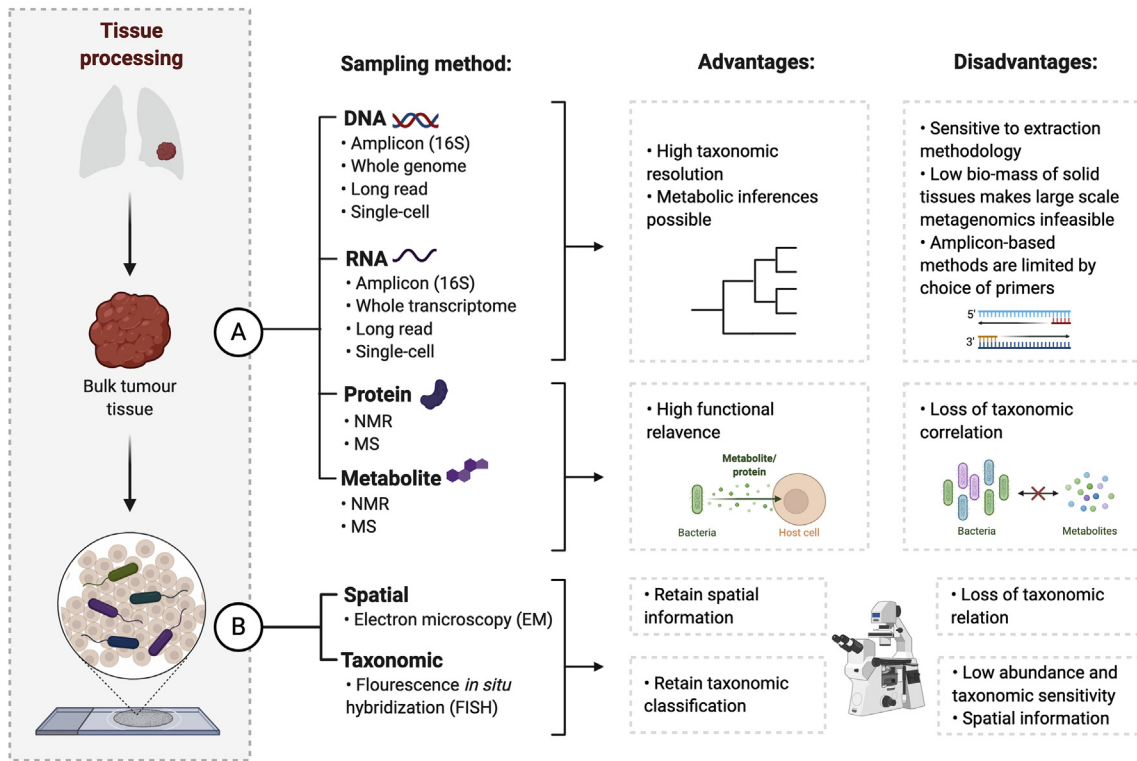
Fig. 4. Methods of capturing microbial information from tumour tissue. Methods of profiling the solid tissue microbiome range from nucleic acid profiling to imaging techniques, all with distinct advantages and disadvantages. A) From bulk tissues, nucleic acid profiling provides taxonomic detail, but is subject to tissue extraction and amplification biases. Further, bulk tissue profiling of metabolites and proteins present in the sample has high functional relevance to bacterial effects on host, but lacks direct relations to taxonomic information. B) Imaging techniques retain spatial detail of tissue, but lack detailed taxonomic information to infer taxonomic-dependent bacterial effect on host.

outnumbered by human reads, shotgun profiling methods are infeasible on a large scale. Studies have shown that ~90% reads from bulk sequencing processes are human, requiring sequencing depth that would be unattainable on a large scale (Human Microbiome Project Consortium 2012; Human Microbiome Project Consortium 2012). Thus, amplification-based methods of profiling are often preferred, such as 16S

rDNA. While this provides a high degree of taxonomic information, it is limited in the selection of primers, and diversity encoded in the 16S rRNA gene (Gohl et al.,2016). Furthermore, as is with all methods of nucleic acid profiling, the acquired information is sensitive to the extraction method used (Sinha et al.,2017).

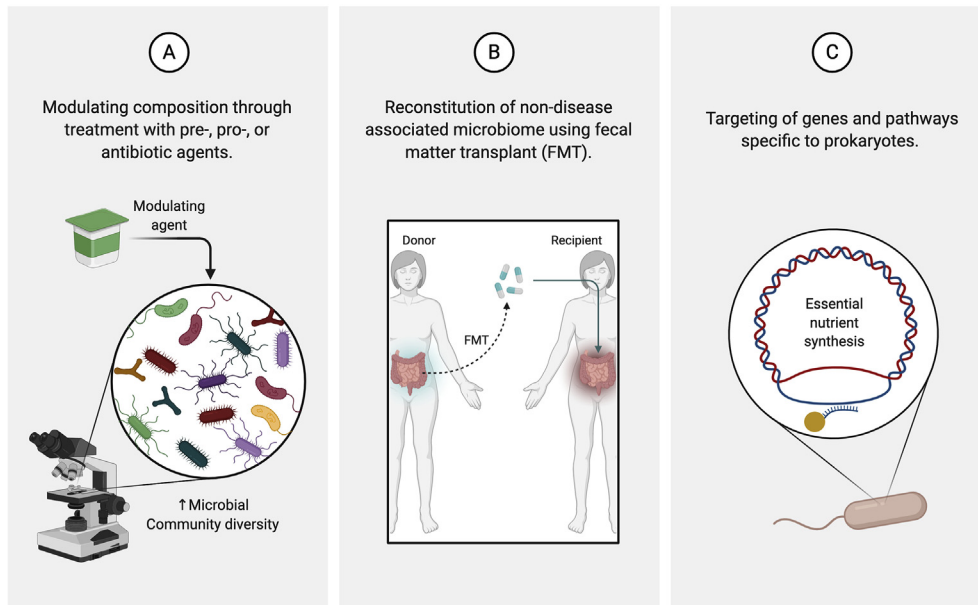Other methods with less emphasis on taxonomy provide information

Fig. 5. Manipulating the microbiome: Potential for cancer therapy. The microbiome represents a new potential therapeutic avenue. A) Bacterial community modulators, including pre-, pro- and anti-biotic agents could be used to alter the microbiome in a therapeutically-beneficial manner. B) Reconstitution of a favorable microbiome via fecal matter transplant (FMT) could aid in tumour-specific colonization at the site. C) The targeting of genes unique to the bacterial community (not present in humans) may increase the potential dosing while limiting side-effects to human cells.

not attainable by sequencing approaches (Mark Welch et al.,2016). Protein and metabolite-based information provide high functional relevance to their effect on the host cells, but the ability to relate to taxonomy is low, as delineation is dependent on the limited resources available about the bacterial metabolic state through the gene annotation of bacterial genomes. Additionally, microscopic and *in situ* techniques allow us to study how these bacteria may be affecting cells in the TME (Fig. 4B). Indeed, information by these techniques has recently revealed that bacteria are present not only in the stroma, but also within tumour cells. However, microscope-based techniques alone provide little taxonomic information needed for targeting specific bacteria.

5. Manipulating the microbiome: Potential for cancer therapy and prevention

Current state of research suggests that knowledge of the microbial population could be harnessed for cancer therapy. The microbiome presents as a source of untapped potential for developing novel therapeutic and preventative strategies, both in the gut directly and through colonization of the tissue-resident bacterial community. Recent studies have examined co-treatment of cancer patients with chemotherapy and antibiotics to alter disease progression with success (Morgun et al.,2015). In addition to using antibiotic treatment to inhibit selected microbial populations, modulation of the microbiome can also be achieved through other means, for example, using pre- and/or pro-biotic agents to shape population composition (Fig. 5A). Further, both cross-patient and autologous fecal matter transplant models have been shown as efficacious in restoring gut microbiome health - in the cancer context, restoring the microbiome after ablation by chemotherapy (Fig. 5B) (Wu et al., 2019). As microbes are evolutionarily distanced from humans, it presents unique opportunities for targeting non-conserved genes; for example, pathway components in bacterial metabolism, such as genes for essential amino acid biosynthesis that are absent in human cells (Fig. 5C). While the field of targeting of the microbiome for therapy in cancer is only in its infancy, the evaluation of the microbiome in human tumours represents a promising novel angle to improve cancer detection, response to therapy, and patient survival.

Contribution statement

Erin A Marshall: Conceptualization, investigation, writing – original draft, visualization. **Nikita Telkar:** Writing – review and editing, visualization. **Wan L Lam:** Conceptualization, writing – review and editing, supervision, funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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