Environmental insults and compensative responses

when microbiome meets cancer

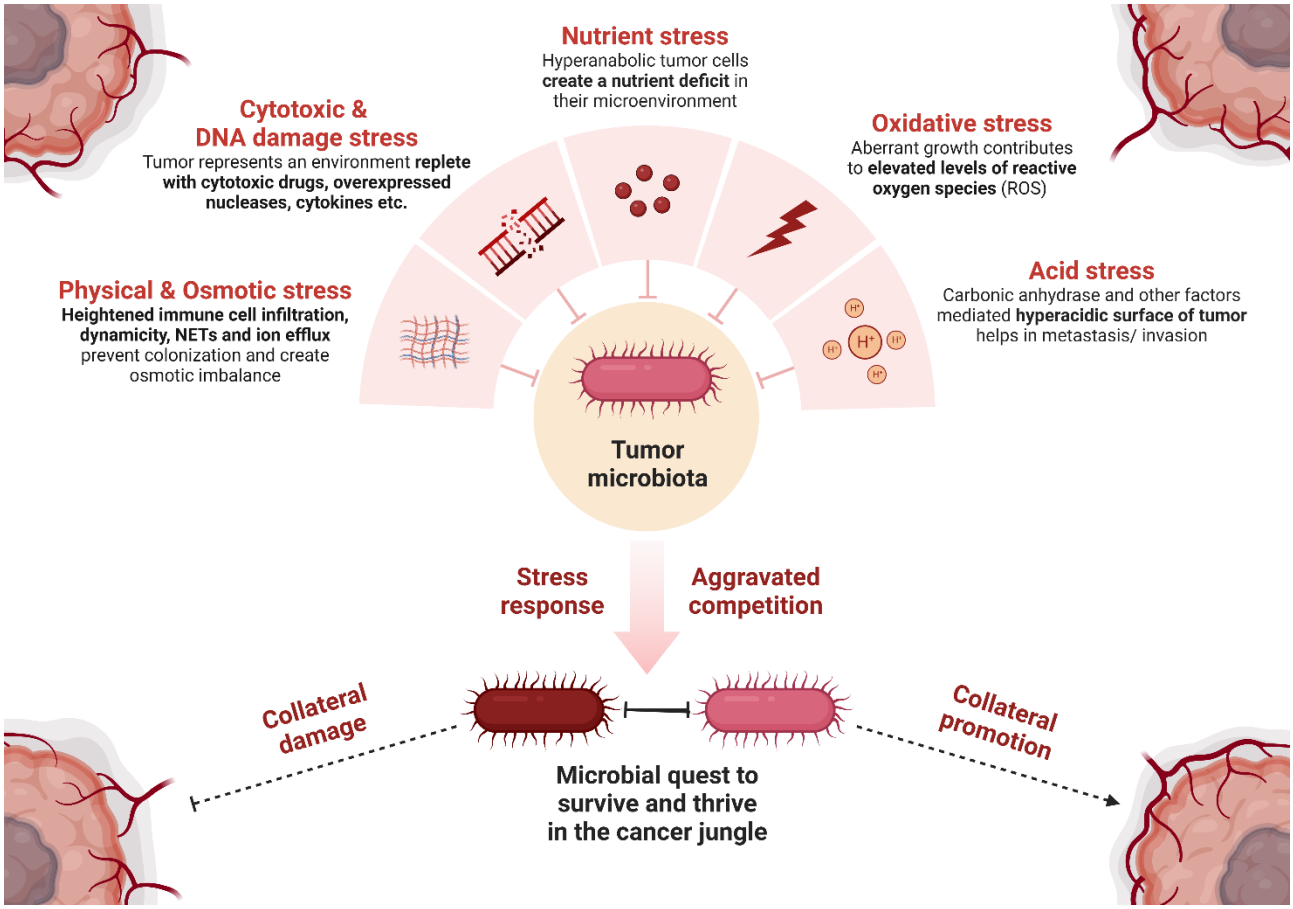
Sunil Nagpal[1,2,3*] and Sharmila S. Mande[1*]

[1]TCS Research, Tata Consultancy Services Ltd, Pune, India – 411013

[2]CSIR-Institute of Genomics and Integrative Biology (CSIR-IGIB), New Delhi-110025, India

[3]Academy of Scientific and Innovative Research (AcSIR), Ghaziabad- 201002, India

*Corresponding author. Email: sunil.nagpal@tcs.com, sharmila.mande@tcs.com



Abstract

Tumor microenvironment has recently been ascribed a new hallmark – the polymorphic microbiome. Accumulating evidence regarding the tissue specific territories of tumor-microbiome have opened new and interesting avenues. A pertinent question is regarding the functional consequence of the meeting of the host-microbiome with cancer. Given microbial communities have predominantly been explored through an ecological perspective, it is important that the foundational aspects of ecological stress and the fight to 'survive and thrive' are accounted for tumor-micro(b)environment as well. Building on existing evidence and classical microbial ecology, here we attempt to characterize the ecological stresses and the compensative responses of the microorganisms inside the tumor microenvironment. What insults would microbes experience inside the cancer jungle? How would they respond to these insults? How the interplay of stress and microbial quest for survival would influence the fate of tumor? This work asks these questions and tries to describe this underdiscussed ecological interface of the tumor and its microbiota. It is hoped that a larger scientific thought on the importance of microbial competition sensing vis-à-vis tumor-microenvironment would be stimulated.

1. Introduction – the cancer-microbe interface

Microbial association with oncopathology has been discussed for decades, with reports of anti-cancerous activity of bacterial toxins dating back to a century ago [1]. Discovery of specific microorganisms inside various tumors and their causal associations have consistently been reported for past several decades [2,3]. However, it was not until recently that a successful and comprehensive characterization of the microbiome associated with different human tumor types was achieved at a large scale (amassing more than 1500 samples) [4]. It laid the foundation for what may be termed as the tissue specific territories of tumor microbiome. Importantly, the breakthrough quashed many prevailing doubts pertaining to the contamination linked discoveries [4,5]. Several reports characterizing the intratumoral microbiota have now emerged in the last 3 years alone, consolidating the existence and importance of the tumor micro(b)environment [4,6–18]. Previously, reports of success in building an onco-diagnostic tool using tissue and blood associated microbial-signatures in treatment-naive cancer patients had also ignited interest towards looking into the sparse microbial content of the tumors [19]. While these pioneering studies provide guiding evidence towards differential microbial community compositions in and around cancer cells [4] and preference of the microbes to inhabit microniches [18], the functional models for tumor associated ‘communities of microbes’ warrant further research.

Success of colonization of tumors by microbes is expected to depend primarily on two factors (i) an influx of the micro-organisms, and (ii) availability of conducive conditions for them to survive, thrive and co-exist in the tumor microenvironment. While the influx can be driven by factors like luminal infiltrations (Figure 1) through compromised epithelial/mucosal barrier [20,21], inheritance from normal adjacent tissues or NAT [4], zipper/trigger mechanisms of bacterial invasion [22] and circulatory contributions from leaky vasculature of the tumor [23,24], survival/thrival/co-existence is not only dependent on the availability of favourable micro-niches in the tumor microenvironment

[18] but also on the activation of microbial stress responses against the perceived unfavourable 'environmental insults' (including the inter/intraspecies competition).

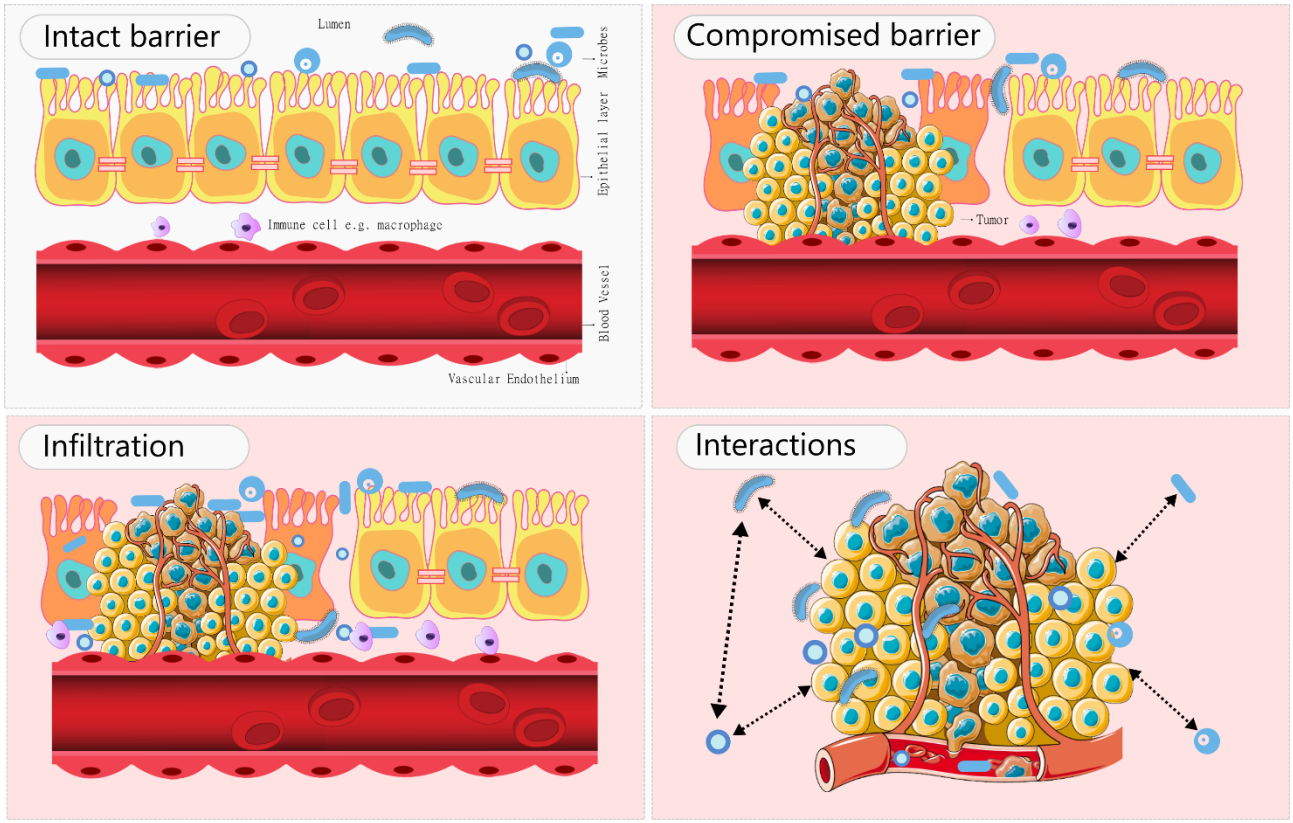


Figure 1. Graphical representation of a scenario, showcasing events that can contribute to intratumoral microbiome. Once the host-microbiota enters the jungle of tumor micro-environment, its quest for surviving and thriving begins (represented by microbe-tumor/microenvironment and microbe-microbe interactions).

2. The environmental insults inside the tumor microenvironment

Tumor microenvironment in fact offers several challenges/insults to the visiting microbes as summarised in the graphical abstract and the Figure 2. These include –

2.1. Nutrient Stress

Two key hallmarks of tumor are the hyperproliferation and hyperanabolism [25]. The unregulated proliferation leads to heightened energy and anabolic needs [25,26]. Consequently, the tumor-microenvironment is always nutrient deprived. While the adaptively programmed cancer cells are always hungry for glucose to utilize it 'effectively and rapidly' through the Warburg effect [26,27], the oncogenic mutations generally lead to a heterogenous

cancerous mass dependent on 'not one but various limiting substrates', leading to a continuous pressure on a variety of nutrients in the milieu of the tumor [26]. This is further aggravated in the Cancer stem cells (CSCs) which represent a subpopulation in the tumor microenvironment, and are undifferentiated and highly aggressive [28]. The infiltrating and intratumor microorganisms are therefore expected to encounter a perpetually hungry and aggressive competitor as soon as they enter the tumor-microenvironment. How the visitors (microbes) would respond to this nutrient stress, can potentially guide the development of meaningful functional models of the tumor-micro(b)environment. Notably, the necrotic regions in the tumor however represent an exception, offering a less competitive, nutrient rich hypoxic microniche for the growth and proliferation of the microorganisms [18,29].

2.2. Oxidative stress

Reactive oxygen species (ROS), the free radicals, bearing unpaired reactive electron in their valence shells, are normal byproducts of cellular respiration (oxidative phosphorylation). Redox homeostasis is critical for maintaining a balance between the reactive oxygen species (ROS) and antioxidants [30]. This antioxidant-enzymes (e.g. superoxide dismutase or SOD) mediated redox balance prevents the normal cells from cytotoxic damage and checks the tumorigenic effects of ROS as well [30]. The balance of redox homeostasis however doesn't prevail in the tumor microenvironment which is replete with the ROS (the oxidative stress) due to hyperproliferation, hyper-metabolism, mitochondrial dysfunction, infiltrating immune cells, genetic (oncogenic) alterations, upregulated oxidases, peroxisome activity and among more [31]. While primarily tumorigenic, ROS can inhibit tumors as well owing to their cytotoxic nature [30,31]. Cancer cells therefore employ adaptive metabolic modes of managing the high ROS levels through NADPH accumulation, glutamine and folate metabolism etc [31]. The incoming microorganisms would also need independent intrinsic mechanisms to fend this insult off or perish due to the deleterious effects of free radicals on

various macromolecules (DNA, proteins, lipids and more), including an eventual cell death. The collateral impact of said adaptive mechanisms on the tumor (microenvironment) would be interesting to probe and understand.

2.3. Physical and Osmotic stress

Tumors are like wounds that never heal [32]. Unlike normal tissues with a stable structure, composition and biochemistry, tumor microenvironment is highly dynamic and unstable. This dynamicity is attributed to the continuous angiogenesis, leaky vasculature, plasma extravasation, a progression towards desmoplasia or solid tumors, among more [32]. Furthermore, the compressive stress faced by solid tumors while invading and navigating through the normal adjacent tissue, causes increased intracellular tonicity (osmotic pressure), triggering the upregulation of sodium efflux by tumors into the TME [33]. Consequently, tumoral microbes are expected to face significant (i) *mechanical stress* due to the dynamic spatio-temporal composition of tumor, preventing surface attachment or promoting detachment, hence challenging the colonization of the TME and (ii) *osmotic stress* due to the efflux of ions challenging microbial survival under the perturbed osmo-homeostasis. The continuous infiltration of inflammatory and immune cells [32,34], including macrophages and neutrophils, in the never healing wounds of tumor, can further aggravate the physical stress on the microbes seeking a firm attachment or colonization. A notable example of immune surveillance mediated physical stress pertains to the expression of neutrophil extracellular traps (NETs) in the tumor microenvironment [35]. NETs are extracellular complexes containing fibres of decondensed chromatin (DNA), decorating protein granules, antimicrobial proteins and histones used as a self-sacrificing defence mechanism (NETosis) by the neutrophils to trap and kill invading microbial pathogens too large to engulf [36]. There are mixed evidence towards the impact of NETs on tumors. Studies have indicated an anti-cancer role of NETs through apoptosis, necrosis, ROS and H_2O_2 mediated cytotoxicity [35].

Evidence are also accumulating that tumors are more inclined to leverage the NETs for proliferation and micro-metastasis [37,38]. It is however invariably well-founded that NETs function to inhibit or kill invading microbes. The strategies adopted by microbes to adapt against or address these environmental stresses interfering with colonization would therefore be additionally critical in understanding the microbe-tumor interplay, especially from a spatio-temporal standpoint.

2.4. Acid stress

The Warburg-effect or the preference for glycolytic metabolism is known to lower the pH of tumor-microenvironment [27,39]. This is attributed to the rapid extrusion of accumulated lactate to the extracellular environment. Additionally, the acidosis is also promoted by the membrane-bound carbonic anhydrases through the release of protons while sequestering carbon dioxide [39]. Both these acidification promoting mechanisms are essentially 'adaptive responses' of the cancer cells towards heightened energy needs (glycolytic metabolism) and hypoxia (over expressed carbonic anhydrases). As a result, tumor-microenvironment exhibits an inverted pH gradient ($pH_{extracellular} < pH_{intracellular}$), opposite to the normal tissues/cellular environments, where extra-celluar pH is higher than the intracellular pH. An alkaline intracellular pH helps tumors to continue proliferate and evade apoptosis within the physiological pH range (7.2-7.4), while an acidic microenvironment (6.3-7.0) enables activation of proteases and metastatic pathways, enabling cellular dispersion, immune-evasion, drug-resistance, and invasion of healthy tissues [39]. Given the heterogenous nature of tumors, a stable pH gradient cannot be expected in the tumor-microenvironment. Moreover, the steepness in the pH changes between the normal cellular environment and the tumor-microenvironment can also be dictated by the biogeography of the host (e.g. normal extracellular pH in: airway mucosa ~ 5.5-7.9, stomach ~ 1.5-3.5, colon: 6.1-7.5) [40,41]. It would be interesting to understand how the dynamic, slightly acidic pH environment of

tumors can affect the survival of the infiltrating microbes, which can have diverse pH sensitivities. The acidosis driven dispersion/metastasis of cancer cells can additionally exert a physical stress on the existing colonies or the microbes seeking a site of attachment [39,42]. Tumor-associated pH gradients and associated heterogeneity can therefore potentially influence colonization and subsequent interactions between the tumor and the microbiome, warranting further research.

2.5. Xenobiotic and DNA damage stress

In addition to the intrinsic hallmarks of cancer offering a variety of stresses to the visiting microbiota, the extrinsic interventional regimens exert tremendous stress on the tumor, normal tissues, and the native microbiome in and beyond tumor-microenvironment. Cytotoxic and inhibitory effects of the xenobiotic chemotherapeutic agents on microbes, much of which are attributed to the DNA damaging traits of these chemicals, are infact well founded [43,44]. Given that antibiotics have consistently been employed in many chemotherapies for their anti-cancer properties, the DNA damaging/inhibitory/microbicidal action of the chemotherapeutic regimens are rather expected [45]. Maier and colleagues however also demonstrated, through in-vitro studies, the inhibitory effects of even the non-antibiotic chemotherapeutic agents on well-known commensal microorganisms of the human gut [46]. It has also been recently proven that even the conventional myelosuppressive chemotherapy disrupts intestinal microbiome [47]. The heterogeneity added to the tumor-microenvironment by the (often) harsh therapeutic regimens, is therefore expected to add to the insults faced by the visiting microbes. Understanding the microbial response towards exposure to this stressful microenvironment replete with the chemotherapeutic agents can not only (potentially) describe the ecological basis of the consolidation of tumor-microbiome, but also the microbe-drug-tumor interplay.

Furthermore, microbial genetic material can also be stressed by the ROS (as described earlier) and the pool of nucleases expressed in the tumor-microenvironment. Nucleases, the enzymes that can hydrolyse nucleic acids, have consistently been perceived as promising biomarkers for cancer. This is attributed to their frequently observed overexpression, with some reports of interindividual variability, in the cancers of various types [48]. Nucleases however are also critical towards establishing innate immunity against bacteria and viruses. This is achieved through pattern recognition receptor (PRR) mediated pathways, which are aberrantly expressed in tumors [49]. These nucleic acid degraders, ranging from exonucleases to endonucleases, are known to be expressed intracellularly, extracellularly as well as 'on the membrane' of cancer cells, marking their omnipresence in the tumor-microenvironment (Yang 2011). While the functional significance of the largely overexpressed tumoral nucleases remain to be fully understood, studies have associated the overexpression of nucleases like Flap endonuclease1 (FEN1), Human apurinic/apyrimidinic endonuclease1 (APE1), Excision repair cross-complementing group 1 xeroderma pigmentosum complementation group F (ERCC1-XPF), Three prime repair exonuclease (TREX2), and more with aggravated tumor growth and digressive response to chemotherapy (poor prognosis and survival) [48]. Nucleases can also have bacterial origin, predominantly employed in the bacterial warfare for survival in the competitive environments, targeting the non-self microbes and host cells. Regardless of their origin, nucleases can target the genetic material and other accessible nucleic acids of the tumoral microbiome, exposing them to heightened DNA damage stress and immune surveillance. Microbial response to these multipronged stresses on their genetic material is an important factor deserving attention, for an overall functional understanding of tumor-microbiome's response to its meeting with the cancer.

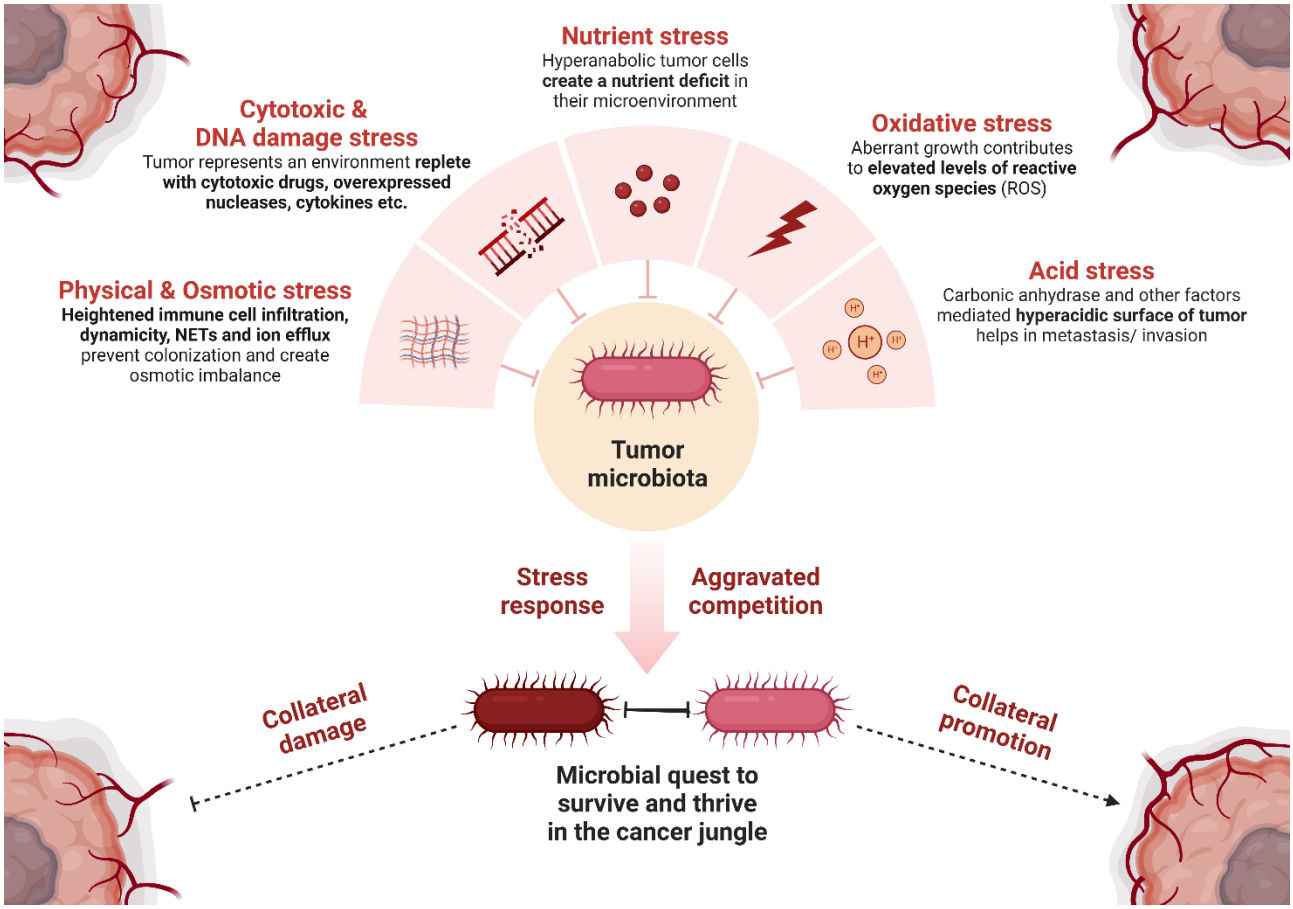


Figure 2. Characterization of the key environmental insults offered by tumor-microenvironment to the infiltrating/intratumoral microbes. Nutrient stress, oxidative stress, acid stress, physical & osmotic stress and DNA damaging/cytotoxic stress in combination are expected to offer significant and persistent insults to the incoming/prevailing microbes in the tumor microenvironment. Microbial response to these stresses may in collateral promote or damage the tumor cells.

3. Responding to the insults – microbial (counter) interactions

Microorganisms have evolved over billions of years to develop regulatory machineries for mitigating the environmental stresses through well-orchestrated gene regulatory networks [51]. The stringent stress response and the general stress response are two key well-founded hallmarks of the stress regulatory responses in microbes [51,52]. Depending upon the nature of stress 'perceived', as described in the subsequent sections, microbes can switch to an appropriate response mechanism for survival. Survival (and resilience) however is a function of 'facilitation' under a harsh environment and 'persistence' through the complex intra/interspecies interactions (competition/cooperation) [53].

This is also described by Chesson in the species co-existence theory, attributing a stabilized community structure to the influence of the environment on inter/intraspecies interactions including the consequent tolerance of invaders/stabilized community to the mutual competition [54,55]. The competitive phenotypes of microbes broadly fall into two categories - (i) interference phenotypes and (ii) exploitative phenotypes [56,57]. Interference competition occurs when the ability of a microbe to survive or attain resources is directly thwarted by interfering phenotypes or antagonistic interactions like chemical warfare and contact dependent-killing. Production of broad-spectrum antibiotics and strain-specific bacteriocins to eliminate rival microorganisms is a typical example of this chemical warfare mediated interference competition [56,58]. Exploitative competition on the other hand is an indirect competition, experienced when microbes attempt to survive in a resource limited environment among competitors with overlapping nutrient requirements [56]. This entails phenotypes like secretion of nutrient-harvesting molecules (e.g. siderophores for iron sequestration), upregulation of transport or uptake pathways, secretion of digestive proteases/nucleases and even secretion of toxins like bacteriocins to specifically inhibit microorganisms with overlapping nutrient needs [52,56,57]. An insight into the competition sensing mechanisms in the microorganisms in fact rationally indicates that exploitative competition generates the interference competition between the microbes, with the larger goal of ruling out any contest for the resources by adopting strategies which can inhibit, displace, or kill the competitors [52]. As Cornforth and Foster propose, an umbrella term of "competition sensing" is less restrictive. It allows an emphasis on the ability of the microbes to sense any harmful stimulus or stressor, perceiving its origins in potential competitors, self or non-self [52]. The suitability and strength of the response to the perceived stimuli would therefore dictate the fate and function(s) of a microbial ecosystem. Given the heterogenous nature of tumor-microenvironment, the dynamics governing the multi-species stress response and competition under the harsh/variable environment of cancer [54,55] potentially hold an important key to understand tumor-microbe interplay. Simply put, the balance of ***'the stress, the stress response and survival'*** in

the tumor micro(b)environment can govern the dynamics of crosstalk between ***'the cancer and the microbes'.*** Notably though, despite the microbial stress response being defensive and compensative in nature, it may not necessarily inhibit the cause of stress, i.e., cancer. This is unlike the response against competing microorganisms, where one microbe or community tries to win against the other (the world of microbe-kills-microbe). The composition of microbial community, density of the microbial populations, tumor physiology, the nature and the quantum of the evoked microbial stress response and the immunological response against microbial invasion is expected to decide the anti-tumor or tumorigenic role of the tumor microbiome.

For simplicity in describing the overarching theme of this article (environmental insults and compensative responses), bacterial ecology and stress response mechanisms will primarily be emphasized in the subsequent sections. The terms 'microbes and bacteria' would therefore be used interchangeably. Bacteria after all are prolifically studied microorganisms offering well founded and valuable models for understanding microbial response to environmental stresses.

3.1. Doing collateral damage - Tumor targeting response of microbes

The stringent stress response (SSR) is an evolutionary conserved specific stress response mechanism, mediated by the alarmone 'guanosine tetraphosphate (ppGpp)', that allows bacteria to reprogram their transcriptional activities when faced with nutrient stress (particularly amino-acid, fatty acid and iron limitations) [59,60]. This entails a switch from translation and biosynthesis to upregulated accumulation of limited resources [52,59]. The state of nutrient stress offered by hyper anabolic cancer cells, aggravated by the overlapping nutrient requirements of the tumoral microbes, can evoke the SSR in the tumor-microbiota. This can reciprocate nutrient stress on cancer (Figure 3), limiting its proliferation by competing for the nutrients critical for tumor progression, particularly BCAA, acetate and iron [61–63]. Ecologically, a quasi-exploitative competition between the microbes sensing the competitive nutrient environment can elicit secretion of antimicrobial peptides/toxins like bacteriocins and other antibiotics. These

microbiome derived molecules, primarily produced to fend off the perceived competition from the microbes with overlapping nutrient requirements, subject to the thriving of a favourable microbial community, may potentially inhibit the cancer cells in collateral damage (Figure 3A) [64,65]. A significantly high production of colicins and microcins (anti-cancer bacteriocins) by mucosal microbiome in CRC patients provides encouraging evidence in this regard [66]. The evidence pertaining to the ability of bacteriocins to cross epithelial and vascular endothelial cells add to the plausibility of a targeted response not only by the intra-tumoral microbes, but by the luminal, mucosal, NAT or stromal microbiome as well [67].

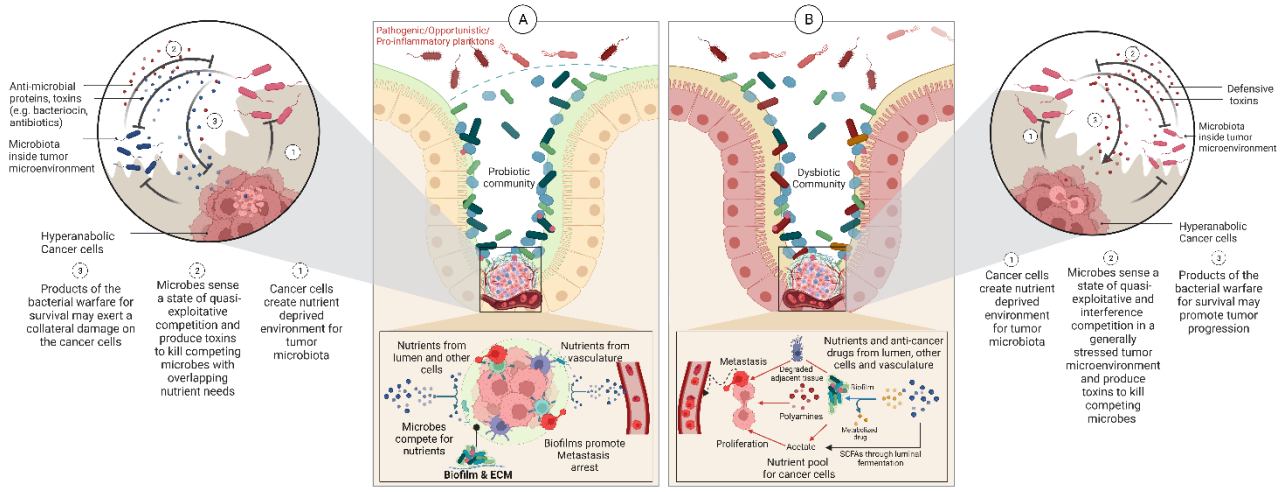


Figure 3. Microbial response to nutrient limited and stressed tumor microenvironment

(A) Microbes may inflict **c**ollateral damage on the tumor cells through competitive uptake of nutrients by the tumor microbiota. Expression of nutrient stress linked phenotype (e.g. Biofilms) can aggravate the nutrient stress on hyperanabolic tumor cells and can also prevent dispersion of cancer stem cells to other tissues (metastasis arrest). A quasi-exploitative competition between the microorganisms may also ensue, leading to production of antimicrobial peptides/toxins (e.g. bacteriocins) to thwart competition from microbes with overlapping nutrient needs. Prevalence of a probiotic microbial community in and around the tumor microenvironment is expected to cause more collateral damage to cancer than a (dysbiotic) community of pathobionts. (B) Biofilms inside the tumor microenvironment, produced in response to the nutrient stress can favor cancer proliferation and metastasis through polyamine biosynthesis, degradation of therapeutic drugs and disruption of normal adjacent tissue. Competition for survival between pathobionts (due to a dysbiotic native community) may lead to upregulation of pro-inflammatory microbial toxins, further promoting the cancer progression.

The presence of a global 'General Stress Response (GSR)' mechanism in bacteria, is however a key weapon in their arsenal of defence against a broad range of environmental insults [68]. It is mediated by the specialized transcriptional sigma (σ) factor(s) that compete with the house keeping sigma factor to redirect transcription towards hundreds of prokaryotic stress response genes, collectively called the general stress regulon. [68,69]. Physio-biochemical stresses triggering the expression of this regulon are rather well founded. These include bacterial exposure to nutrient starvation, free radicals, heat, osmotic imbalance, acids, alcohols, membrane & DNA damaging environmental stimuli and more that (threaten to) compromise the integrity/survival of a microbial cell [69]. Given the association of GSR with a regulon consisting of hundreds of compensative genes, the phenotypic output of this defence mechanism is multi-pronged and confers a broad cross-resistance against a variety of rather unrelated stresses [68]. Accumulation of nutrients (e.g. glycogen, amino acids, acetate, iron etc), shift to fermentation and biofilm formation, expression of enzymes like catalases and oxidoreductases, accumulation or synthesis of osmoprotectants (e.g. trehalose, amino acids, K^+), heightened expression of 'amino acid decarboxylases, deaminases, proton pumping, biofilm formation' for acid tolerance are few classical examples of GSR phenotypes [68–73]. It is also pertinent to note the association of GSR with transition to the stationary growth phase which is marked by a metabolic switch to the accumulation of inhibitory by-products/secondary metabolites like antibiotics, toxins and even complex behaviours like biofilm formation [68].

The diverse environmental insults offered by tumor microenvironment to the visiting/thriving microbes are expected to trigger the expression of aforementioned general stress regulon. This is particularly true for nutrient and oxidative stress (abundantly prevailing in the TME) which are known to confer a broad cross-protectivity through the activation of general stress response [69]. Table 1, backed by literature evidence, is compiled to describe the the key GSR linked phenotypic outcomes that can (potentially) inflict a collateral reciprocation of insults on the cancer cells. The

312 relevant tumorigenic/tumor-promoting outcomes of the said GSR expression are summarised in
313 the Figure 4 and in the subsequent sections of this article.

314

GSR target	Phenotype	Mechanism of collateral damage for cancer	Reference
Nutrient stress	• Accumulation of nutrients (e.g. glycogen, amino acids, acetate, iron etc) • shift to fermentation • biofilm formation	• Resource limitation for hyperanabolic cancer cells (cancers need glycogen, acetate, iron, BCAA etc) • anti-mitotic role of SCFAs • metastasis distraction by biofilms through secretion of exopolysaccharides, preventing cancer cell binding to the endothelial cells	[70,74–76]
Oxidative stress	• Expression of free radical scavenging enzymes like catalases, oxidoreductases, Superoxide dismutase • Damage repairing proteins like thioredoxins, glutaredoxins, and methionine sulfoxide reductases	• Free radical clearance and release of damage repairing proteins limits DNA damage, inflammatory cytokines, metastasis, and oncogenic mutagenesis	[31,77]
Acid Stress	• expression of amino acid (Arginine and Glutamate) decarboxylases • activation of Arginine deaminase system • proton pumping • increased glycolytic activity • biofilm formation	• Cancer cells are arginine addict (deprivation leads to cancer cell death) • Glutamate is a key substrate for cancer cells • Proton release by intra-tumor microbes can disrupt pH of cancer cells • Heightened microbial glycolytic activity and biofilm formation can compete for energy metabolism and prevent metastasis	[26,39,78]

Physical Stress	• upregulated MSCRAMMs* and biofilm formation • expression of autolysin like enzymes and release of eDNA*, teichoic acid and other cytoplasmic contents • upregulation of virulence factors like surface endonucleases	• MSCRAMMs mediate covalent binding leading to persistent biofilms that can compete for nutrition and arrest metastasis • eDNA and teichoic acids can mediate non-covalent binding of microbes to cancer, and can also trigger immune surveillance for collateral recognition of cancer cells • Degradation of NETs* and other entrapments by surface endonucleases can prevent metastasis	[36,37,42, 79,80]
Osmotic stress	• Solute uptake including amino acids, potassium ions (K^+) • Synthesis and accumulation of Trehalose	• Uptake of amino acids as solutes can limit cancer energy metabolism • Trehalose released through mechanosensitive channels and upon bacterial lysis can reduce inflammation, limit free radicals, enhance apoptosis • Uptake of the storm of K^+ ions released by dying cancer cells can limit suppression of cancer killing T-cell effector function	[61,81,82]
DNA damage	SOS response upregulates – • biofilms with (drug resistant) persister population • Intraspecies competition and consequent toxin secretion • Toxin-anti toxin (TA) system activation • Horizontal gene transfer	• Persistent biofilms can compete for energy metabolism and prevent metastasis • Toxins against intraspecies competition (e.g. colicins) can inhibit cancers • TA systems can specifically cause cancer cell death (e.g. MazF-MazE toxin–antitoxin of E.coli against pancreatic and colorectal cancers) • Anti-cancer toxins/antibiotics encoded by plasmids can promote population level phenotype through HGT	[52,64,83 –86]

** MSCRAMM: **M**icrobial **S**urface **C**omponents **R**ecognizing **A**dhesive **M**atrix **M**olecules are microbial surface proteins that adhere specifically to host extra-cellular matrix (ECM)*; eDNA: extracellular DNA; NETs: Neutrophil Extracellular Traps

Table 1. Potential responses of tumor invading/inhabiting microbiota mediated by the expression of GSR regulon under diverse environmental insults of the TME. Notably, the GSR targets can be ameliorated by any stressor that can activate entire general stress regulon (conferring cross protectivity).

Under the right tumor microbial composition (native or interventional), this GSR and stationary phase linked in-vivo production of compensative products may even support cancer-therapy by priming the onco-immune system towards anti-tumor effects. The reported role of intra-tumoral probiotic gut-microbes in facilitating immunotherapy through the secondary metabolite mediated triggering of the STING signalling (stimulator of interferon genes), highlights this significance of tumoral colonization by commensal bacteria like *Bifidobacterium sp.* [87].

From an ecological point of view, insults like oxidative stress, DNA damage stress, physical stress and acid stress are perceived as instances of direct challenges interfering with the ability of the microbes to survive and thrive. This calls for an activation of interference competitive phenotype and hence release of antibiotics and strain-specific bacteriocins towards the microbe-kill-microbe response [56]. The collateral damage inflicted on the cancer cells by this chemical warfare started by microbes under the perceived interference competition is plausible and therefore deserves exploration. The molecular mechanistic details underpinning this warfare may be described by the evolutionary matured stress response mechanisms as described earlier. In addition to the development of functional models, this would be important for the design of live biotherapeutics or dietary interventions aiming to favourably customize the microbial and metabolite composition of tumor invading/prevailing microbiota. **Figure 4** provides a graphical overview of the (aforementioned) events that may ensue in tumor micro(b)environment.

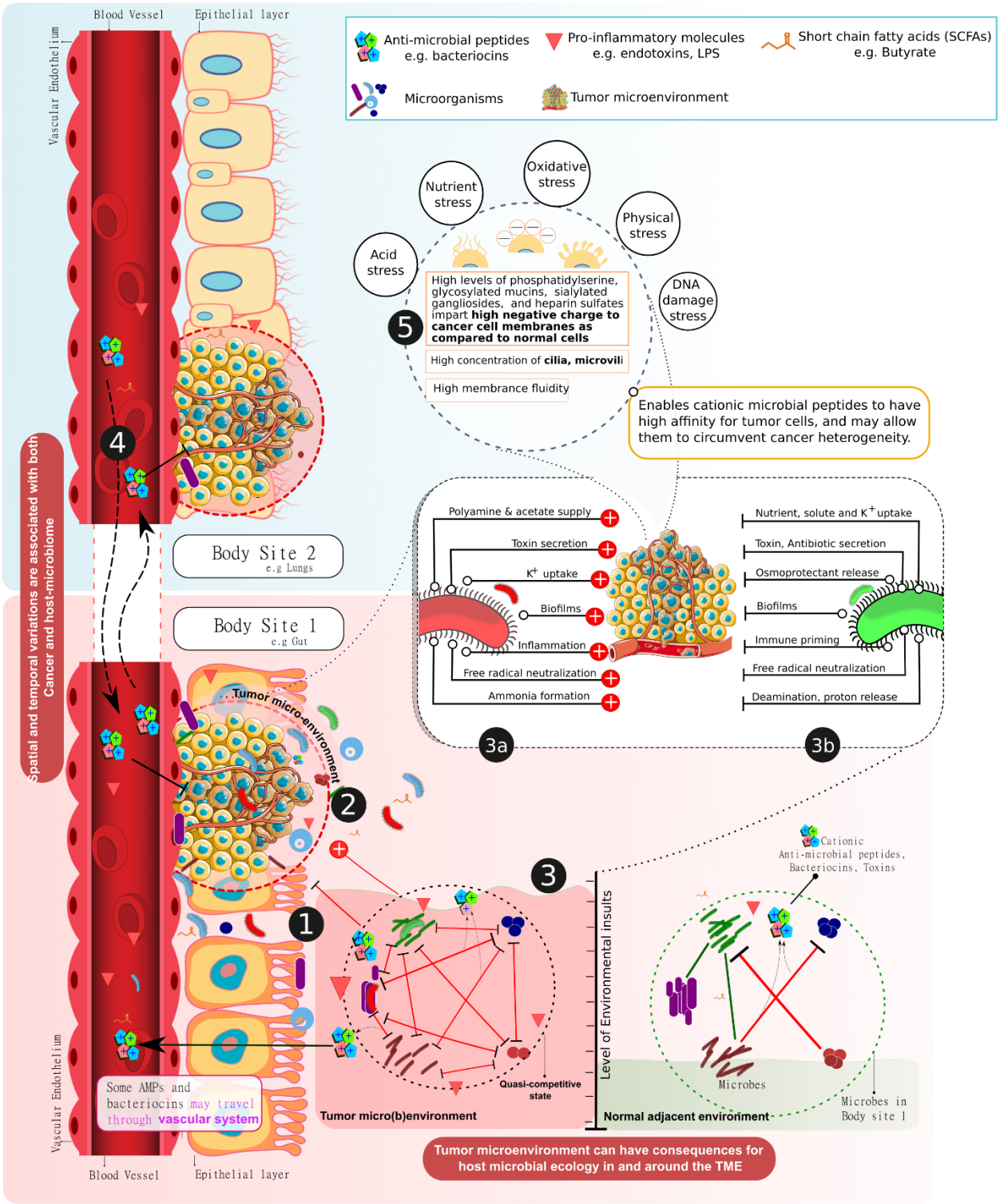


Figure 4. Microbial quest for survival in the tumor affected ecosystem.

Microbes and microbial products may infiltrate to tumors through dysfunctional epithelial barrier, adjacent tissues or circulatory system. (2) Stressful tumor environment can trigger Stringent and general stress response in microbes. (3) Environmental insults can lead to quasi-exploitative and quasi-interference competition between tumor microbes. (3a) Competitive environment and resultant stress response manifests in the form of upregulation of nutrient and ion uptake,

synthesis of anti-microbial peptides/toxins, shift to fermentation, biofilm formation, redox balance and more causing collateral damage to cancer. (3b) Microbial responses can be oncogenic/promoting too (e.g. toxin secretion, polyamine metabolism, ammonia formation, inflammatory LPS, lowered oxidative stress on tumor, potassium influx). (4) Microbial metabolites including toxins and AMPs can access circulatory system for potential systemic effects. (5) Properties of cancer cell membranes can enable targeted attack by cationic anti-microbial peptides

3.2. Into the wounds that never heal - Tumor promoting response of microbes

While Rudolph Virchow first linked chronic inflammation with tumor development [88], Harold Dvorak's comparison of tumors with the 'Wounds that never heal' notified similarities between tumor stroma generation (essential for tumor growth) and wound healing [32]. Microbial invasion of these wounds can spur the inflammation process [89], supporting the tumor elicited inflammation characterised by an accelerated recruitment of immune cells and up-regulation of pro-inflammatory cytokines and growth factors [89–92]. This can not only promote tumor progression but also aggravate the associated adverse symptoms. Notably, in-addition to the immune-regulating components of microbial anatomy like flagellin and lipopolysaccharide (LPS), the secondary metabolic products of microbial stress response like toxins (e.g. colibactin, endotoxin) can be pro-inflammatory and oncogenic [3,89,90,93]. These, as interjected earlier, are expected to be elicited in response to the diverse environmental insults faced by the invading microorganisms (Table 1).

The responses controlled by the general stress regulon may additionally support tumor progression (Figure 4). This includes - (i) the neutralization of oxidative stress by microbes in the tumor microenvironment, thereby lowering the compensative load on tumor cells which are also sensitive to redox imbalance [31,77] (ii) acid stress management by microbial urease system leading to the formation of normally cytotoxic, proinflammatory but a potent nitrogen-reservoir for cancer cells - ammonia [73,94] (iii) influx of potassium ions upon activation of osmotic stress response in the microbes, lowering intracellular tonicity of tumors and limiting T-cell stemness

that enables cancer clearance [33,95,96] and (iv) the reported role of stress resilient bacterial biofilms, a phenotypic response expected against nutrient, physical, DNA damage and acid stress, in initiation and progression of cancer through polyamine metabolism, toxin secretion and other well founded pro-oncogenic responses is worth consideration as well (Figure 3B) [97]. Furthermore, the fermentative state of microbial growth under anoxic and nutrient depleted environment of tumors and normal adjacent tissue (e.g. gut epithelium and lumen) may contribute acetate (the most abundant SCFA), which, even though is reported for its anticancer potential, is also a key energy molecule for proliferating cancer cells (Figure 3B) [62].

Unsurprisingly, the molecular basis of ecological interactions of tumor prevailing/invading microbes with the potentially insulting environmental conditions dictate that the meeting of microbes with cancer can have both deleterious and advantageous consequences for the tumor. Where the balance would weigh more, can only be determined by the stabilized (or tweaked) microbial population and its functional potential. It is therefore important, as we next discuss, to ponder over the directions that can branch out of this school of thought and potential limitations in assuming the native microbial populations of tumors, including any microbe-tumor cross-talk.

4. Future directions and limitations

Human body essentially serves as an ecosystem to the colonizing microbes. The organ and tissue specific (spatio-temporal) territories of host microbiome are governed by the myriad of physiological, physical, metabolic and nutritional conditions specific to the sites of microbial colonization. Tumor development needs to be viewed as an ecological disturbance and its micro-environment as a perturbed niche capable of reshaping the structure of individual microbial populations through systemic and localized environmental pressures. How prevailing microbiota responds and survives against the ecological stresses offered by tumor development/progression is expected to drive the compositional and metabolic variations observed in different individuals, across different types of tumors. Such an understanding is critical to drive the development of in-silico models of tumor

micro(b)environment through due attention to the dynamics of underlying metabolic fluxes and multi-species interactions (‘host-microbe, tumor-microbe, microbe-microbe and even tumor-tumor’). A functional gradation and classification of key microbial players (e.g. drivers, passengers) identified inside the tumor micro-environment may enable validation of the well founded driver-passenger models of various types of cancer [98,99]. Importantly, the functional understanding of microbial response to tumor micro-environment can aid development of therapeutic regimens aimed at modulating microbial populations and function thereof inside and around the cancer. This includes, but not limited to the probiotic and prebiotic formulations that can assist an accelerated reshaping of host and tumor microbiome towards an ‘anti-cancer’ community [66,100,101].

Cancer however is a complex disease characterised not only by abnormally dividing hyper-anabolic cells, unique micro-environment and location or site-specific manifestations but multifactorial confounders like specialized care and aggressive therapeutic regimens (e.g. chemotherapy, radiotherapy) etc [102–104]. This mileu of confounding factors can significantly impact the systemic as well as the localized host microbial ecology which may not overlap with the expected or characteristic response of microbes inside and in vicinity of a treatment-naive tumor environment**.** Addtionally, the personalized nature of host microbiome, governed by spatio-temporal dynamics adds to the complexity of factors that need to be accommodated for arriving at in-silico models or translatable interventions. The systemic implications of surgical (like Ostomy) and case-dependent dosages and durations of invasive therapeutic regimens like radiation or chemotherapy only add to the associated complications of the disease and its ecosystem [102,105,106]. Worth consideration are the challanges associated with reproducing the results of microbiome studies (the reproducibility crisis), especially considering the composionally sparse microbiota of tumor [4]. Given the extremely low microbial load of tumor associated samples, contaminants become an additional and key bottleneck to address against innumerable sources of contamination throughout the lengthy workflow of microbiome study. Nevertheless, under all these variables, microbial

response to stimuli is a significant constant and deserves attention for any research that intends to decipher the functional models of cancer micro(b)environment.

5. Conclusion

Surviving and thriving are key to organismal existence in the living world, microbes are no exception. Appreciating the challenges associated with colonization of an environment as complex and heterogeneous as tumor and linking them with what is well founded in microbial ecology can drive foundational understanding of microbial role in modulating the tumor microenvironment. Here an effort was made to characterize the relevant stresses in the tumor microenvironment that may serve as insults compromising the colonization and survival of microbes in the harsh environment of the tumors. Upon revisiting the classical evidence of microbial ecology/competition and stress response, it becomes encouragingly clear that collateral impact of microbial compensative responses to the consistent insults of the TME could hold an important key for developing functional models of tumor-microbe interaction. The success of various dietary regimens and microbial interventions (e.g. pre/probiotics), that attempt to channelize the host-microbial arsenal for cancer prevention or treatment may after all have roots in the basic concept of microbial competition sensing and their response to the environmental stimuli[52,107,108]. Understanding such stimuli in tumor micro(b)environment and microbial responses to the same, is critical to throw light on what happens (and can happen), when microbiota meets cancer.

Abbreviations

NAT: Normal Adjacent Tissue; **GSR**: General Stress Response/Regulon; **SSR**: Stringent Stress Response; **BCAA**: Branched Chain Amino Acids; **SCFA**: Short Chain Fatty Acids; **STING**: Stimulator of Interferon Genes; **LPS**: Lipopolysaccharide

Declaration

No part of this article was written or designed with the aid of any automated or generative tool like ChatGPT or DALL.E. All ideas and content of this work are authors' own work, including a sincere effort to manually go through various research articles that served as reference or evidence towards building the presented perspective(s).

Author contribution

S.N. conceived the idea, wrote the manuscript draft and designed figures; S.S.M. supervised the work; Both the authors edited, reviewed and approved the submitted manuscript

Acknowledgement

Authors would like to thank Tata Consultancy Services Research for promoting the spirit of fundamental and applied research. Authors gratefully acknowledge the "angiogenesis" PNG from Servier Medical Art by Servier, licensed under a Creative Commons Attribution 3.0 Unported License. Figure 2 and Figure 3 were designed with Biorender.com.

Competing interests

Authors are salaried research Scientists at TCS Research. TCS holds a portfolio of patents in oncology, microbiome, nutrition and more.

Funding

Both the authors are salaried research Scientists at TCS Research.

References

1 Coley WB (1910) The Treatment of Inoperable Sarcoma by Bacterial Toxins (the Mixed Toxins of the Streptococcus erysipelas and the Bacillus prodigiosus). *Proc R Soc Med* **3**.

2 Parida S & Sharma D (2021) The microbiome and cancer: Creating friendly neighborhoods and removing the foes with in A C. *Cancer Res* **81**.

3 Wong-Rolle A, Wei HK, Zhao C & Jin C (2021) Unexpected guests in the tumor microenvironment: microbiome in cancer. *Protein Cell* **12**.

4 Nejman D, Livyatan I, Fuks G, Gavert N, Zwang Y, Geller LT, Rotter-Maskowitz A, Weiser R, Mallel G, Gigi E, Meltser A, Douglas GM, Kamer I, Gopalakrishnan V, Dadosh T, Levin-Zaidman S, Avnet S, Atlan T, Cooper ZA, Arora R, Cogdill AP, Khan MAW, Ologun G, Bussi Y, Weinberger A, Lotan-Pompan M, Golani O, Perry G, Rokah M, Bahar-Shany K, Rozeman EA, Blank CU, Ronai A, Shaoul R, Amit A, Dorfman T, Kremer R, Cohen ZR, Harnof S, Siegal T, Yehuda-Shnaidman E, Gal-Yam EN, Shapira H, Baldini N, Langille MGI, Ben-Nun A, Kaufman B, Nissan A, Golan T, Dadiani M, Levanon K, Bar J, Yust SK, Barshack I, Peeper DS, Raz DJ, Segal E, Wargo JA, Sandbank J, Shental N & Straussman R (2020) The human tumor microbiome is composed of tumor type-specific intracellular bacteria. *Science (1979)* **368**.

5 Robinson KM, Crabtree J, Mattick JSA, Anderson KE & Hotopp JCD (2017) Distinguishing potential bacteria-tumor associations from contamination in a secondary data analysis of public cancer genome sequence data. *Microbiome* **5**.

6 Atreya CE & Turnbaugh PJ (2020) Probing the tumor micro(b)environment. *Science (1979)* **368**.

7 Choi JK, Naffouje SA, Goto M, Wang J, Christov K, Rademacher DJ, Green A, Stecenko AA, Chakrabarty AM, das Gupta TK & Yamada T (2023) Cross-talk between cancer and Pseudomonas aeruginosa mediates tumor suppression. *Commun Biol* **6**, 16.

8 Yuan L, Yang P, Wei G, Hu X, Chen S, Lu J, Yang L, He X & Bao G (2022) Tumor microbiome diversity influences papillary thyroid cancer invasion. *Commun Biol* **5**, 864.

9 Hermida LC, Gertz EM & Ruppin E (2021) Analyzing the tumor microbiome to predict cancer patient survival and drug response. *Cancer Res* **81**.

10 Feng Z, Hu Y, Wang X, Li Y, Yu Y, He J, Li H, Zhang T, Zhang L, Shen G & Ding X (2022) In situ imaging for tumor microbiome interactions via imaging mass cytometry on single-cell level. *Cytometry Part A* **101**.

11 Thyagarajan S, Zhang Y, Thapa S, Allen MS, Phillips N, Chaudhary P, Kashyap M v. & Vishwanatha JK (2020) Comparative analysis of racial differences in breast tumor microbiome. *Sci Rep* **10**.

12 Murphy CL, Barrett M, Pellanda P, Killeen S, McCourt M, Andrews E, O' Riordain M, Shanahan F & O'Toole P (2021) Mapping the colorectal tumor microbiota. *Gut Microbes* **13**.

13 Okuda S, Shimada Y, Tajima Y, Yuza K, Hirose Y, Ichikawa H, Nagahashi M, Sakata J, Ling Y, Miura N, Sugai M, Watanabe Y, Takeuchi S & Wakai T (2021) Profiling of host genetic alterations and intra-tumor microbiomes in colorectal cancer. *Comput Struct Biotechnol J* **19**.

14 Bahig H, Fuller CD, Mitra A, Yoshida-Court K, Solley T, Ping Ng S, Abu-Gheida I, Elgohari B, Delgado A, Rosenthal DI, Garden AS, Frank SJ, Reddy JP, Colbert L & Klopp A (2021)

Longitudinal characterization of the tumoral microbiome during radiotherapy in HPV-associated oropharynx cancer. *Clin Transl Radiat Oncol* **26**.

15 Livyatan I, Nejman D, Shental N & Straussman R (2020) Characterization of the human tumor microbiome reveals tumor-type specific intra-cellular bacteria. *Oncoimmunology* **9**.

16 Guo W, Zhang Y, Guo S, Mei Z, Liao H, Dong H, Wu K, Ye H, Zhang Y, Zhu Y, Lang J, Hu L, Jin G & Kong X (2021) Tumor microbiome contributes to an aggressive phenotype in the basal-like subtype of pancreatic cancer. *Commun Biol* **4**.

17 Zwinsová B, Petrov VA, Hrivňáková M, Smatana S, Micenková L, Kazdová N, Popovici V, Hrstka R, Šefr R, Bencsiková B, Zdražilová-Dubská L, Brychtová V, Nenutil R, Vídeňská P & Budinská E (2021) Colorectal tumour mucosa microbiome is enriched in oral pathogens and defines three subtypes that correlate with markers of tumour progression. *Cancers (Basel)* **13**.

18 Niño JLG, Wu H, LaCourse KD, Kempchinsky AG, Baryiames A, Barber B, Futran N, Houlton J, Sather C, Sicinska E, Taylor A, Minot SS, Johnston CD & Bullman S (2022) Effect of the intratumoral microbiota on spatial and cellular heterogeneity in cancer. *Nature*.

19 Poore GD, Kopylova E, Zhu Q, Carpenter C, Fraraccio S, Wandro S, Kosciolek T, Janssen S, Metcalf J, Song SJ, Kanbar J, Miller-Montgomery S, Heaton R, Mckay R, Patel SP, Swafford AD & Knight R (2020) Microbiome analyses of blood and tissues suggest cancer diagnostic approach. *Nature* **579**.

20 Mullin JM (2004) Epithelial barriers, compartmentation, and cancer. *Sci STKE* **2004**.

21 Soler AP, Miller RD, Laughlin K v., Carp NZ, Klurfeld DM & Mullin JM (1999) Increased tight junctional permeability is associated with the development of colon cancer. *Carcinogenesis* **20**.

22 O Cróinín T & Backert S (2012) Host epithelial cell invasion by Campylobacter jejuni: trigger or zipper mechanism? *Front Cell Infect Microbiol* **2**.

23 Cummins J & Tangney M (2013) Bacteria and tumours: Causative agents or opportunistic inhabitants? *Infect Agent Cancer* **8**.

24 Hashizume H, Baluk P, Morikawa S, McLean JW, Thurston G, Roberge S, Jain RK & McDonald DM (2000) Openings between defective endothelial cells explain tumor vessel leakiness. *American Journal of Pathology* **156**.

25 Hanahan D (2022) Hallmarks of Cancer: New Dimensions. *Cancer Discov* **12**.

26 Sullivan MR & vander Heiden MG (2019) Determinants of nutrient limitation in cancer. *Crit Rev Biochem Mol Biol* **54**.

27 Liberti M v. & Locasale JW (2016) The Warburg Effect: How Does it Benefit Cancer Cells? *Trends Biochem Sci* **41**.

28 Yadav UP, Singh T, Kumar P, Sharma P, Kaur H, Sharma S, Singh S, Kumar S & Mehta K (2020) Metabolic Adaptations in Cancer Stem Cells. *Front Oncol* **10**.

29 Zhou S, Gravekamp C, Bermudes D & Liu K (2018) Tumour-targeting bacteria engineered to fight cancer. *Nat Rev Cancer* **18**.

30 Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M & Telser J (2007) Free radicals and antioxidants in normal physiological functions and human disease. *International Journal of Biochemistry and Cell Biology* **39**.

31 Hayes JD, Dinkova-Kostova AT & Tew KD (2020) Oxidative Stress in Cancer. *Cancer Cell* **38**.

32 Dvorak HF (2015) Tumors: Wounds that do not heal-redux. *Cancer Immunol Res* **3**.

33 McGrail DJ, McAndrews KM, Brandenburg CP, Ravikumar N, Kieu QMN & Dawson MR (2015) Osmotic Regulation Is Required for Cancer Cell Survival under Solid Stress. *Biophys J* **109**.

34 Flier JS, Underhill LH & Dvorak HF (1986) Tumors: Wounds That Do Not Heal. *New England Journal of Medicine* **315**.

35 de Meo ML & Spicer JD (2021) The role of neutrophil extracellular traps in cancer progression and metastasis. *Semin Immunol* **57**.

564 36 Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y &
565 Zychlinsky A (2004) Neutrophil Extracellular Traps Kill Bacteria. *Science (1979)* **303**.
566 37 Yang L, Liu Q, Zhang X, Liu X, Zhou B, Chen J, Huang D, Li J, Li H, Chen F, Liu J, Xing Y,
567 Chen X, Su S & Song E (2020) DNA of neutrophil extracellular traps promotes cancer
568 metastasis via CCDC25. *Nature* **583**.
569 38 Masucci MT, Minopoli M, del Vecchio S & Carriero MV (2020) The Emerging Role of
570 Neutrophil Extracellular Traps (NETs) in Tumor Progression and Metastasis. *Front Immunol*
571 **11**.
572 39 Lee SH & Griffiths JR (2020) How and why are cancers acidic? Carbonic anhydrase ix and the
573 homeostatic control of tumour extracellular ph. *Cancers (Basel)* **12**.
574 40 Evans DF, Pye G, Bramley R, Clark AG, Dyson TJ & Hardcastle JD (1988) Measurement of
575 gastrointestinal pH profiles in normal ambulant human subjects. *Gut* **29**.
576 41 Fischer H & Widdicombe JH (2006) Mechanisms of acid and base secretion by the airway
577 epithelium. *Journal of Membrane Biology* **211**.
578 42 Otto M (2014) Physical stress and bacterial colonization. *FEMS Microbiol Rev* **38**.
579 43 Maurice CF, Haiser HJ & Turnbaugh PJ (2013) Xenobiotics shape the physiology and gene
580 expression of the active human gut microbiome. *Cell* **152**.
581 44 Johnson NP, Razaka H, Wimmer F, Defais M & Villani G (1987) Toxicity, mutagenicity and
582 drug resistance in Escherichia coli treated with platinum antitumor compounds. *Inorganica*
583 *Chim Acta* **137**.
584 45 Shapiro RS (2015) Antimicrobial-Induced DNA Damage and Genomic Instability in Microbial
585 Pathogens. *PLoS Pathog* **11**.
586 46 Maier L, Pruteanu M, Kuhn M, Zeller G, Telzerow A, Anderson EE, Brochado AR, Fernandez
587 KC, Dose H, Mori H, Patil KR, Bork P & Typas A (2018) Extensive impact of non-antibiotic
588 drugs on human gut bacteria. *Nature* **555**.
589 47 Papanicolas LE, Sims SK, Taylor SL, Miller SJ, Karapetis CS, Wesselingh SL, Gordon DL &
590 Rogers GB (2021) Conventional myelosuppressive chemotherapy for non-haematological
591 malignancy disrupts the intestinal microbiome. *BMC Cancer* **21**.
592 48 Balian A & Hernandez FJ (2021) Nucleases as molecular targets for cancer diagnosis. *Biomark*
593 *Res* **9**.
594 49 Nagi RS, Bhat AS & Kumar H (2014) Cancer: A tale of aberrant PRR response. *Front Immunol*
595 **5**.
596 50 Yang W (2011) Nucleases: Diversity of structure, function and mechanism. *Q Rev Biophys* **44**.
597 51 Foster PL (2005) Stress responses and genetic variation in bacteria. *Mutation Research -*
598 *Fundamental and Molecular Mechanisms of Mutagenesis* **569**.
599 52 Cornforth DM & Foster KR (2013) Competition sensing: The social side of bacterial stress
600 responses. *Nat Rev Microbiol* **11**.
601 53 Hart SP & Marshall DJ (2013) Environmental stress, facilitation, competition, and coexistence.
602 *Ecology* **94**.
603 54 Chesson P (1994) Multispecies Competition in Variable Environments. *Theor Popul Biol* **45**.
604 55 Chesson P (2018) Updates on mechanisms of maintenance of species diversity. *Journal of*
605 *Ecology* **106**.
606 56 Hibbing ME, Fuqua C, Parsek MR & Peterson SB (2010) Bacterial competition: Surviving and
607 thriving in the microbial jungle. *Nat Rev Microbiol* **8**.
608 57 Ghoul M & Mitri S (2016) The Ecology and Evolution of Microbial Competition. *Trends*
609 *Microbiol* **24**.
610 58 Bauer MA, Kainz K, Carmona-Gutierrez D & Madeo F (2018) Microbial wars: Competition in
611 ecological niches and within the microbiome. *Microbial Cell* **5**.
612 59 Irving SE, Choudhury NR & Corrigan RM (2021) The stringent response and physiological roles
613 of (pp)pGpp in bacteria. *Nat Rev Microbiol* **19**.

614 60 Boutte CC & Crosson S (2013) Bacterial lifestyle shapes stringent response activation. *Trends*
615 *Microbiol* **21**.
616 61 Sivanand S & vander Heiden MG (2020) Emerging Roles for Branched-Chain Amino Acid
617 Metabolism in Cancer. *Cancer Cell* **37**.
618 62 Schug ZT, vande Voorde J & Gottlieb E (2016) The metabolic fate of acetate in cancer. *Nat Rev*
619 *Cancer* **16**.
620 63 Manz DH, Blanchette NL, Paul BT, Torti FM & Torti S v. (2016) Iron and cancer: Recent
621 insights. *Ann N Y Acad Sci* **1368**.
622 64 Karpiński TM & Adamczak A (2018) Anticancer activity of bacterial proteins and peptides.
623 *Pharmaceutics* **10**.
624 65 Dobson A, Cotter PD, Paul Ross R & Hill C (2012) Bacteriocin production: A probiotic trait?
625 *Appl Environ Microbiol* **78**.
626 66 Kohoutova D, Forstlova M, Moravkova P, Cyrany J, Bosak J, Smajs D, Rejchrt S & Bures J
627 (2020) Bacteriocin production by mucosal bacteria in current and previous colorectal
628 neoplasia. *BMC Cancer* **20**.
629 67 Dreyer L, Smith C, Deane SM, Dicks LMT & van Staden AD (2019) Migration of Bacteriocins
630 Across Gastrointestinal Epithelial and Vascular Endothelial Cells, as Determined Using In
631 Vitro Simulations. *Sci Rep* **9**.
632 68 Gottesman S (2019) Trouble is coming: Signaling pathways that regulate general stress
633 responses in bacteria. *Journal of Biological Chemistry* **294**.
634 69 Boor KJ (2006) Bacterial stress responses: What doesn't kill them can make them stronger.
635 *PLoS Biol* **4**.
636 70 Dauer P & Lengyel E (2019) New Roles for Glycogen in Tumor Progression. *Trends Cancer* **5**.
637 71 Gottschlich L, Geiser P, Bortfeld-Miller M, Field CM & Vorholt JA (2019) Complex general
638 stress response regulation in Sphingomonas melonis Fr1 revealed by transcriptional analyses.
639 *Sci Rep* **9**.
640 72 Bearson S, Bearson B & Foster JW (1997) Acid stress responses in enterobacteria. *FEMS*
641 *Microbiol Lett* **147**.
642 73 Guan N & Liu L (2020) Microbial response to acid stress: mechanisms and applications. *Appl*
643 *Microbiol Biotechnol* **104**.
644 74 Deberardinis RJ & Chandel NS (2016) Fundamentals of cancer metabolism INTRODUCTION
645 AND OVERARCHING PRINCIPLES. *Adv Sci*.
646 75 Ohara T & Mori T (2019) Antiproliferative Effects of Short-chain Fatty Acids on Human
647 Colorectal Cancer Cells via Gene Expression Inhibition. *Anticancer Res* **39**.
648 76 Sieow BFL, Wun KS, Yong WP, Hwang IY & Chang MW (2021) Tweak to Treat:
649 Reprograming Bacteria for Cancer Treatment. *Trends Cancer* **7**.
650 77 Ezraty B, Gennaris A, Barras F & Collet JF (2017) Oxidative stress, protein damage and repair
651 in bacteria. *Nature Reviews Microbiology 2017 15:7* **15**, 385–396.
652 78 Al-Koussa H, el Mais N, Maalouf H, Abi-Habib R & El-Sibai M (2020) Arginine deprivation: A
653 potential therapeutic for cancer cell metastasis? A review. *Cancer Cell Int* **20**.
654 79 Beiter K, Wartha F, Albiger B, Normark S, Zychlinsky A & Henriques-Normark B (2006) An
655 endonuclease allows Streptococcus pneumoniae to escape from neutrophil extracellular traps.
656 *Curr Biol* **16**, 401–407.
657 80 Ho SS, Michalek SM & Nahm MH (2008) Lipoteichoic acid is important in innate immune
658 responses to gram-positive bacteria. *Infect Immun* **76**.
659 81 S N Chaitanya N, Devi A, Sahu S & Alugoju P (2021) Molecular mechanisms of action of
660 Trehalose in cancer: A comprehensive review. *Life Sci* **269**.
661 82 Eil R, Vodnala SK, Clever D, Klebanoff CA, Sukumar M, Pan JH, Palmer DC, Gros A,
662 Yamamoto TN, Patel SJ, Guittard GC, Yu Z, Carbonaro V, Okkenhaug K, Schrump DS,

663 Linehan WM, Roychoudhuri R & Restifo NP (2016) Ionic immune suppression within the
664 tumour microenvironment limits T cell effector function. *Nature* **537**.
665 83 Žgur-Bertok D (2013) DNA Damage Repair and Bacterial Pathogens. *PLoS Pathog* **9**.
666 84 Podlesek Z & Žgur Bertok D (2020) The DNA Damage Inducible SOS Response Is a Key
667 Player in the Generation of Bacterial Persister Cells and Population Wide Tolerance. *Front*
668 *Microbiol* **11**, 1785.
669 85 Inglis RF, Bayramoglu B, Gillor O & Ackermann M (2013) The role of bacteriocins as selfish
670 genetic elements. *Biol Lett* **9**.
671 86 Shapira S, Boustanai I, Kazanov D, ben Shimon M, Fokra A & Arber N (2021) Innovative dual
672 system approach for selective eradication of cancer cells using viral-based delivery of natural
673 bacterial toxin–antitoxin system. *Oncogene* **40**.
674 87 Shi Y, Zheng W, Yang K, Harris KG, Ni K, Xue L, Lin W, Chang EB, Weichselbaum RR & Fu
675 YX (2020) Intratumoral accumulation of gut microbiota facilitates CD47-based
676 immunotherapy via STING signaling. *Journal of Experimental Medicine* **217**.
677 88 David H (1988) Rudolf Virchow and Modern Aspects of Tumor Pathology. *Pathol Res Pract*
678 **183**.
679 89 Grivennikov SI, Wang K, Mucida D, Stewart CA, Schnabl B, Jauch D, Taniguchi K, Yu GY,
680 Österreicher CH, Hung KE, Datz C, Feng Y, Fearon ER, Oukka M, Tessarollo L, Coppola V,
681 Yarovinsky F, Cheroutre H, Eckmann L, Trinchieri G & Karin M (2012) Adenoma-linked
682 barrier defects and microbial products drive IL-23/IL-17-mediated tumour growth. *Nature* **491**.
683 90 Yang W & Cong Y (2021) Gut microbiota-derived metabolites in the regulation of host immune
684 responses and immune-related inflammatory diseases. *Cell Mol Immunol* **18**.
685 91 Wang K & Karin M (2015) Tumor-Elicited Inflammation and Colorectal Cancer. In *Advances in*
686 *Cancer Research*.
687 92 Hou J, Karin M & Sun B (2021) Targeting cancer-promoting inflammation — have anti-
688 inflammatory therapies come of age? *Nat Rev Clin Oncol* **18**.
689 93 Shalapour S & Karin M (2020) Cruel to Be Kind: Epithelial, Microbial, and Immune Cell
690 Interactions in Gastrointestinal Cancers. *Annu Rev Immunol* **38**.
691 94 Li X, Zhu H, Sun W, Yang X, Nie Q & Fang X (2021) Role of glutamine and its metabolite
692 ammonia in crosstalk of cancer-associated fibroblasts and cancer cells. *Cancer Cell Int* **21**.
693 95 Vodnala SK, Eil R, Kishton RJ, Sukumar M, Yamamoto TN, Ha NH, Lee PH, Shin MH, Patel
694 SJ, Yu Z, Palmer DC, Kruhlak MJ, Liu X, Locasale JW, Huang J, Roychoudhuri R, Finkel T,
695 Klebanoff CA & Restifo NP (2019) T cell stemness and dysfunction in tumors are triggered by
696 a common mechanism. *Science (1979)* **363**.
697 96 Csonka LN (1989) Physiological and genetic responses of bacteria to osmotic stress. *Microbiol*
698 *Rev* **53**.
699 97 Li S, Konstantinov SR, Smits R & Peppelenbosch MP (2017) Bacterial Biofilms in Colorectal
700 Cancer Initiation and Progression. *Trends Mol Med* **23**.
701 98 Garza DR, Taddese R, Wirbel J, Zeller G, Boleij A, Huynen MA & Dutilh BE (2020) Metabolic
702 models predict bacterial passengers in colorectal cancer. *Cancer Metab* **8**.
703 99 Geng J, Song Q, Tang X, Liang X, Fan H, Peng H, Guo Q & Zhang Z (2014) Co-occurrence of
704 driver and passenger bacteria in human colorectal cancer. *Gut Pathog* **6**.
705 100 Hols P, Ledesma-García L, Gabant P & Mignolet J (2019) Mobilization of Microbiota
706 Commensals and Their Bacteriocins for Therapeutics. *Trends Microbiol* **27**.
707 101 Chakrabarty AM (2003) Microorganisms and cancer: Quest for a therapy. *J Bacteriol* **185**.
708 102 Park DS, Robertson-Tessi M, Luddy KA, Maini PK, Bonsall MB, Gatenby RA & Anderson
709 ARA (2019) The goldilocks window of personalized chemotherapy: Getting the immune
710 response just right. *Cancer Res* **79**.
711 103 Boedtkjer E & Pedersen SF (2020) The Acidic Tumor Microenvironment as a Driver of
712 Cancer. *Annu Rev Physiol* **82**.

713 104 de Berardinis RJ & Chandel NS (2016) Fundamentals of cancer metabolism. *Sci Adv* **2**.
714 105 Beamish EL, Johnson J, Shaw EJ, Scott NA, Bhowmick A & Rigby RJ (2017) Loop ileostomy-
715 mediated fecal stream diversion is associated with microbial dysbiosis. *Gut Microbes* **8**.
716 106 Helmink BA, Khan MAW, Hermann A, Gopalakrishnan V & Wargo JA (2019) The
717 microbiome, cancer, and cancer therapy. *Nat Med* **25**.
718 107 Kolodziejczyk AA, Zheng D & Elinav E (2019) Diet–microbiota interactions and personalized
719 nutrition. *Nat Rev Microbiol* **17**.
720 108 Lee C & Longo VD (2011) Fasting vs dietary restriction in cellular protection and cancer
721 treatment: From model organisms to patients. *Oncogene* **30**.
722

Table legends:

Table 1. Potential responses of tumor invading/inhabiting microbiota mediated by the expression of GSR regulon under diverse environmental insults of the TME. Notably, the GSR targets can be ameliorated by any stressor that can activate entire general stress regulon (conferring cross protectivity).

Figure legends:

Figure 1. Graphical representation of a scenario, showcasing events that can contribute to intratumoral microbiome. Once the host-microbiota enters the jungle of tumor micro-environment, its quest for surviving and thriving begins (represented by microbe-tumor/microenvironment and microbe-microbe interactions).

Figure 2. Characterization of the key environmental insults offered by tumor-microenvironment to the infiltrating/intratumoral microbes. Nutrient stress, oxidative stress, acid stress, physical & osmotic stress and DNA damaging/cytotoxic stress in combination are expected to offer significant and persistent insults to the incoming/prevailing microbes in the tumor microenvironment. Microbial response to these stresses may in collateral promote or damage the tumor cells.

Figure 3. Microbial response to nutrient limited and stressed tumor microenvironment

(A) Microbes may inflict **c**ollateral damage on the tumor cells through competitive uptake of nutrients by the tumor microbiota. Expression of nutrient stress linked phenotype (e.g. Biofilms) can aggravate the nutrient stress on hyperanabolic tumor cells and can also prevent dispersion of cancer stem cells to other tissues (metastasis arrest). A quasi-exploitative competition between the microorganisms may also ensue, leading to production of antimicrobial peptides/toxins (e.g. bacteriocins) to thwart competition from microbes with overlapping nutrient needs. Prevalence of a probiotic microbial community in and around the tumor microenvironment is expected to cause more collateral damage to cancer than a (dysbiotic) community of pathobionts. (B) Biofilms inside the tumor microenvironment, produced in response to the nutrient stress can favor cancer proliferation and metastasis through polyamine biosynthesis, degradation of therapeutic drugs and disruption of normal adjacent tissue. Competition for survival between pathobionts (due to a dysbiotic native community) may lead to upregulation of pro-inflammatory microbial toxins, further promoting the cancer progression.

Figure 4. Microbial quest for survival in the tumor affected ecosystem.

Microbes and microbial products may infiltrate to tumors through dysfunctional epithelial barrier, adjacent tissues or circulatory system. (2) Stressful tumor environment can trigger Stringent and general stress response in microbes. (3) Environmental insults can lead to quasi-exploitative and quasi-interference competition between tumor microbes. (3a) Competitive environment and resultant stress response manifests in the form of upregulation of nutrient and ion uptake, synthesis of anti-microbial peptides/toxins, shift to fermentation, biofilm formation, redox balance and more causing collateral damage to cancer. (3b) Microbial responses can be oncogenic/promoting too (e.g. toxin secretion, polyamine metabolism, ammonia formation, inflammatory LPS, lowered oxidative stress on tumor, potassium influx). (4) Microbial metabolites including toxins and AMPs can access

circulatory system for potential systemic effects. (5) Properties of cancer cell membranes can enable targeted attack by cationic anti-microbial peptides