Environmental insults and compensative responses

when microbiome meets cancer

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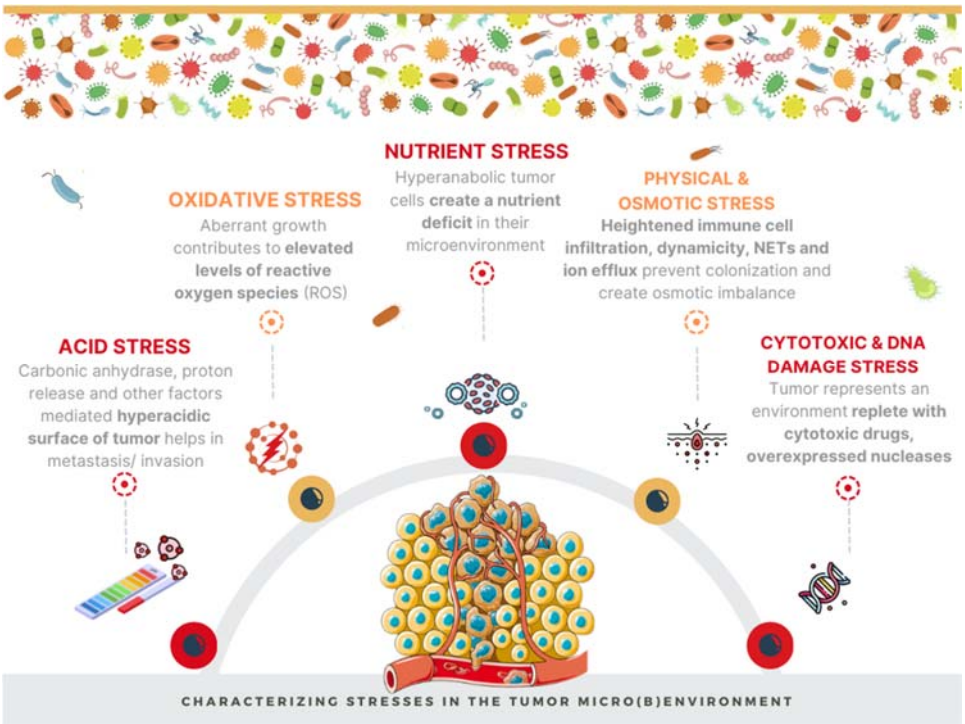
Abstract

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. Understanding the potential events leading to the synapse between the microbiome and the cancer, and characterizing the subsequent environmental insults faced by the (infiltrating and intra-tumoral) microbes is therefore important. Current work, building on existing evidence, aims to characterize the ecological stresses and compensative responses of microbes to describe this underdiscussed ecological interface between tumor and 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).

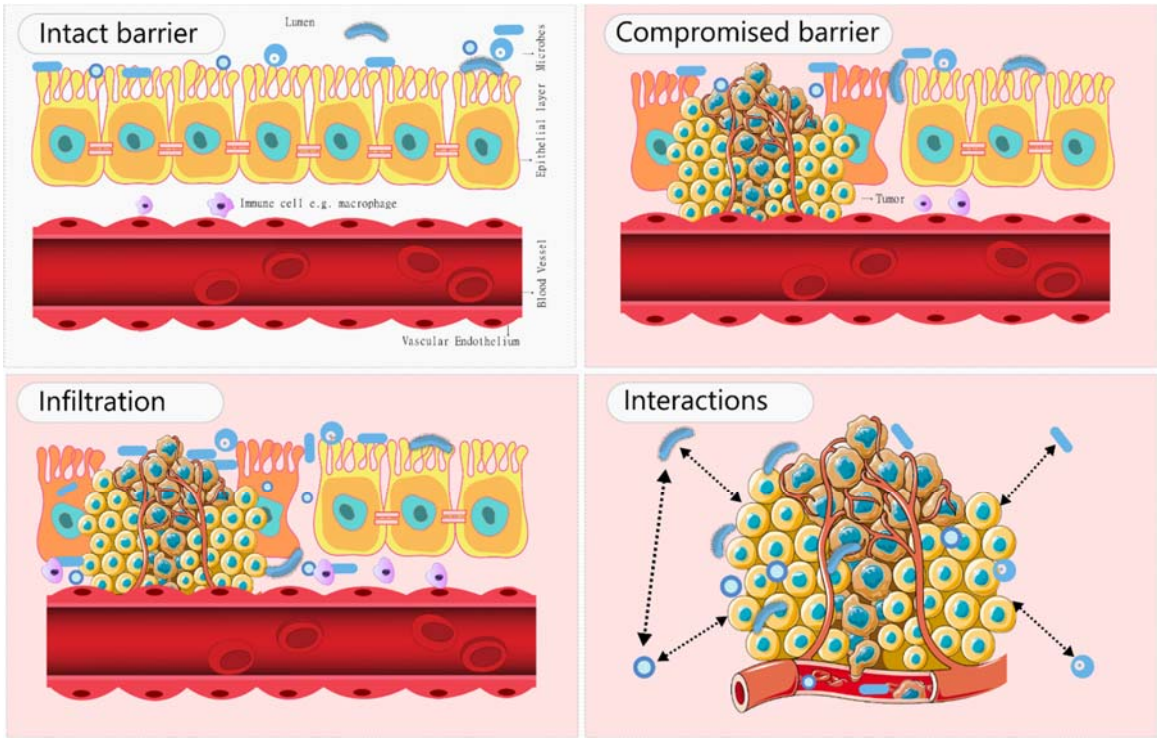


Figure 1. Graphical representation of a scenario, showcasing events that can contribute to intratumoral microbiome. Once the host-microbiota enters the jungle of tumor micro-environment, its quest for surviving and thriving begins (represented by microbe-tumor/microenvironment and microbe-microbe interactions).

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

153 on the existing colonies or the microbes seeking a site of attachment [39,42]. Tumor-associated
154 pH gradients and associated heterogeneity can therefore potentially influence colonization and
155 subsequent interactions between the tumor and the microbiome, warranting further research.

156 ## 2.5. Xenobiotic and DNA damage stress

157 In addition to the intrinsic hallmarks of cancer offering a variety of stresses to the visiting
158 microbiota, the extrinsic interventional regimens exert tremendous stress on the tumor, normal
159 tissues, and the native microbiome in and beyond tumor-microenvironment. Cytotoxic and
160 inhibitory effects of the xenobiotic chemotherapeutic agents on microbes, much of which are
161 attributed to the DNA damaging traits of these chemicals, are infact well founded [43,44]. Given
162 that antibiotics have consistently been employed in many chemotherapies for their anti-cancer
163 properties, the DNA damaging/inhibitory/microbicidal action of the chemotherapeutic
164 regimens are rather expected [45]. Maier and colleagues however also demonstrated, through
165 in-vitro studies, the inhibitory effects of even the non-antibiotic chemotherapeutic agents on
166 well-known commensal microorganisms of the human gut [46]. It has also been recently proven
167 that even the conventional myelosuppressive chemotherapy disrupts intestinal microbiome [47].
168 The heterogeneity added to the tumor-microenvironment by the (often) harsh therapeutic
169 regimens, is therefore expected to add to the insults faced by the visiting microbes.
170 Understanding the microbial response towards exposure to this stressful microenvironment
171 replete with the chemotherapeutic agents can not only (potentially) describe the ecological
172 basis of the consolidation of tumor-microbiome, but also the microbe-drug-tumor interplay.
173 Furthermore, microbial genetic material can also be stressed by the ROS (as described earlier)
174 and the pool of nucleases expressed in the tumor-microenvironment. Nucleases, the enzymes
175 that can hydrolyse nucleic acids, have consistently been perceived as promising biomarkers
176 for cancer. This is attributed to their frequently observed overexpression, with some reports
177 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.

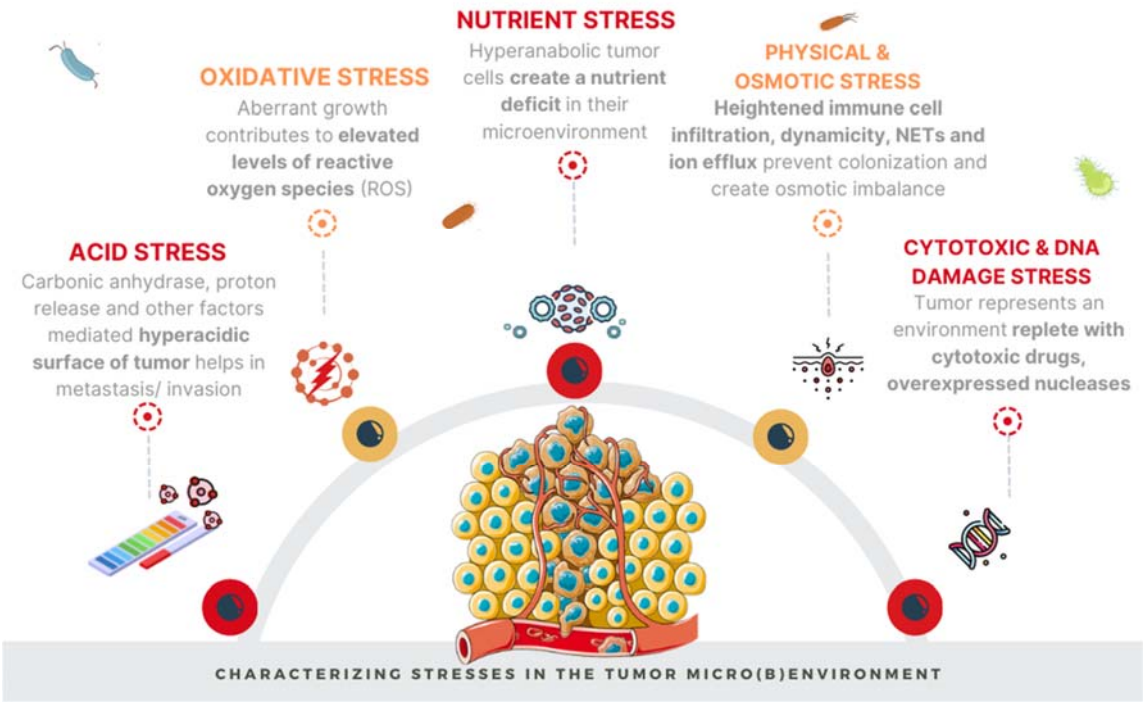


Figure 2. Characterization of the key environmental insults offered by tumor-microenvironment to the infiltrating/intratumoral microbes. Nutrient stress, oxidative stress, acid stress, physical & osmotic stress and DNA damaging/cytotoxic stress in combination are expected to offer significant and persistent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

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, 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 like bacteriocins and other antibiotics. These microbiome derived molecules, primarily produced to fend off the perceived competition from the microbes with overlapping nutrient requirements may potentially inhibit the cancer cells in collateral damage [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].

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

287 particularly true for nutrient and oxidative stress (abundantly prevailing in the TME) which are
288 known to confer a broad cross-protectivity through the activation of general stress response [69].
289 Table 1, backed by literature evidence, is compiled to describe the the key GSR linked phenotypic
290 outcomes that can (potentially) inflict a collateral reciprocation of insults on the cancer cells. The
291 relevant tumorigenic/tumor-promoting outcomes of the said GSR expression are summarised in
292 the Figure 3 and in the subsequent sections of this article.

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

298 priming the onco-immune system towards anti-tumor effects. The reported role of intra-tumoral
299 probiotic gut-microbes in facilitating immunotherapy through the secondary metabolite mediated
300 triggering of the STING signalling (stimulator of interferon genes), highlights this significance of
301 tumoral colonization by commensal bacteria like *Bifidobacterium sp.* [87].

302 From an ecological point of view, insults like oxidative stress, DNA damage stress, physical
303 stress and acid stress are perceived as instances of direct challenges interfering with the ability of
304 the microbes to survive and thrive. This calls for an activation of interference competitive
305 phenotype and hence release of antibiotics and strain-specific bacteriocins towards the microbe-
306 kill-microbe response [56]. The collateral damage inflicted on the cancer cells by this chemical
307 warfare started by microbes under the perceived interference competition is plausible and
308 therefore deserves exploration. The molecular mechanistic details underpinning this warfare may
309 be described by the evolutionary matured stress response mechanisms as described earlier. In
310 addition to the development of functional models, this would be important for the design of live
311 biotherapeutics or dietary interventions aiming to favourably customize the microbial and
312 metabolite composition of tumor invading/prevailing microbiota. **Figure 3** provides a graphical
313 overview of the (aforementioned) events that may ensue in tumor micro(b)environment.

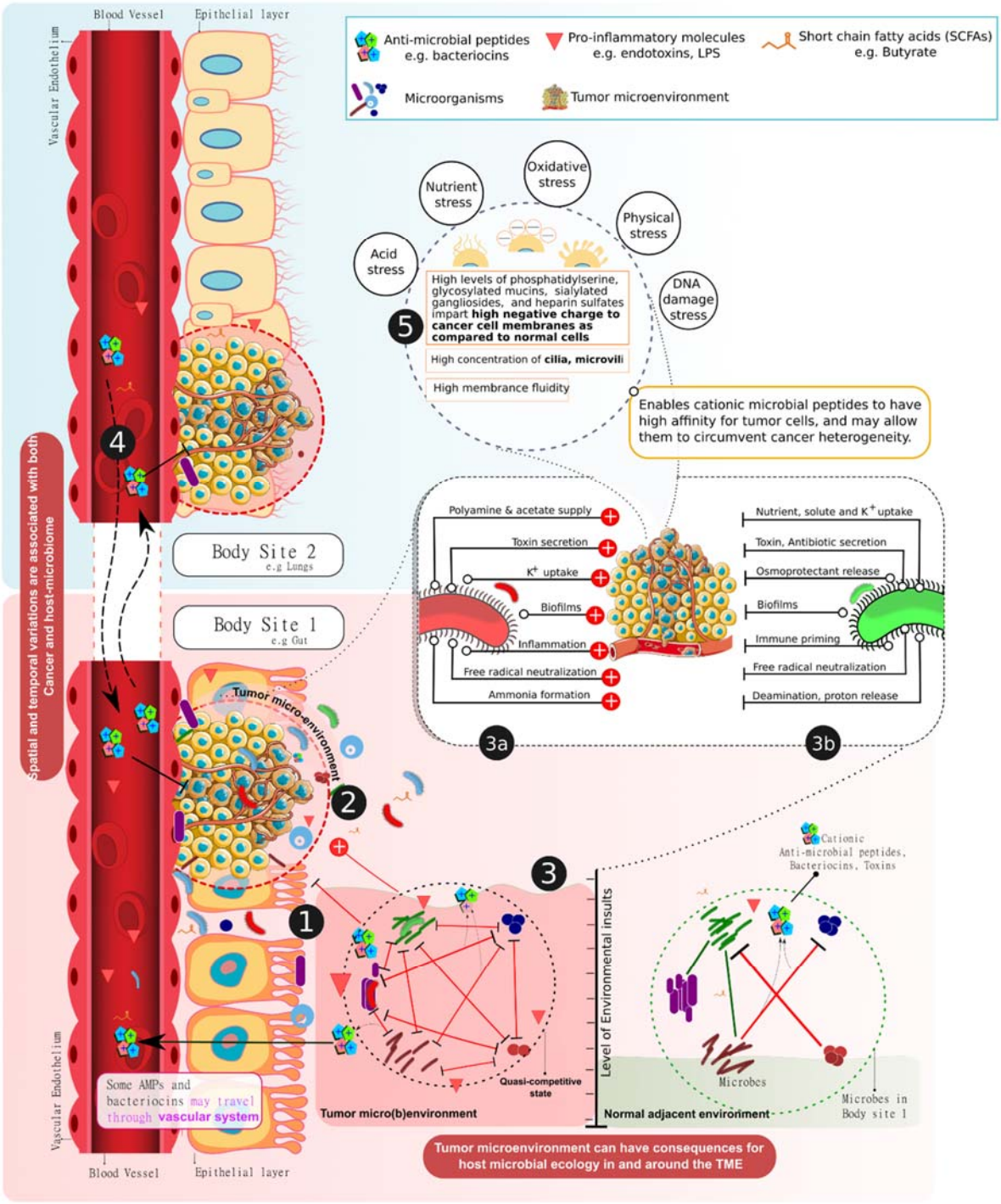


Figure 3. Microbial quest for survival in the tumor affected ecosystem.

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 3). 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 [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 [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

Conceived the idea: SN; Manuscript draft and Figures' design: SN; Supervision: SSM; Proofreading: SSM, SN.

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Competing interests

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

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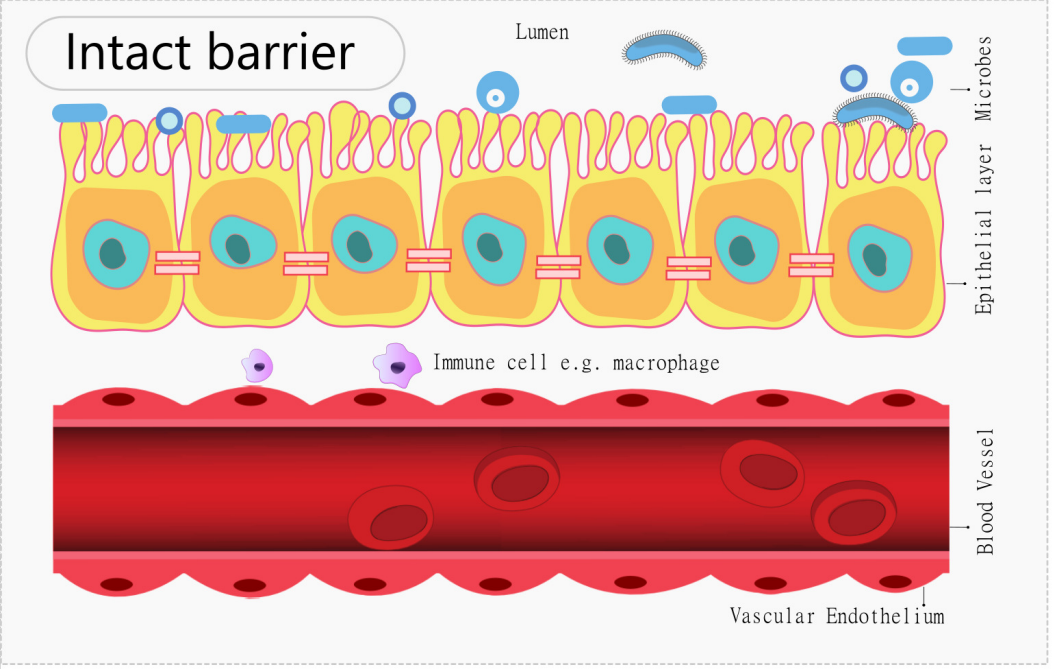
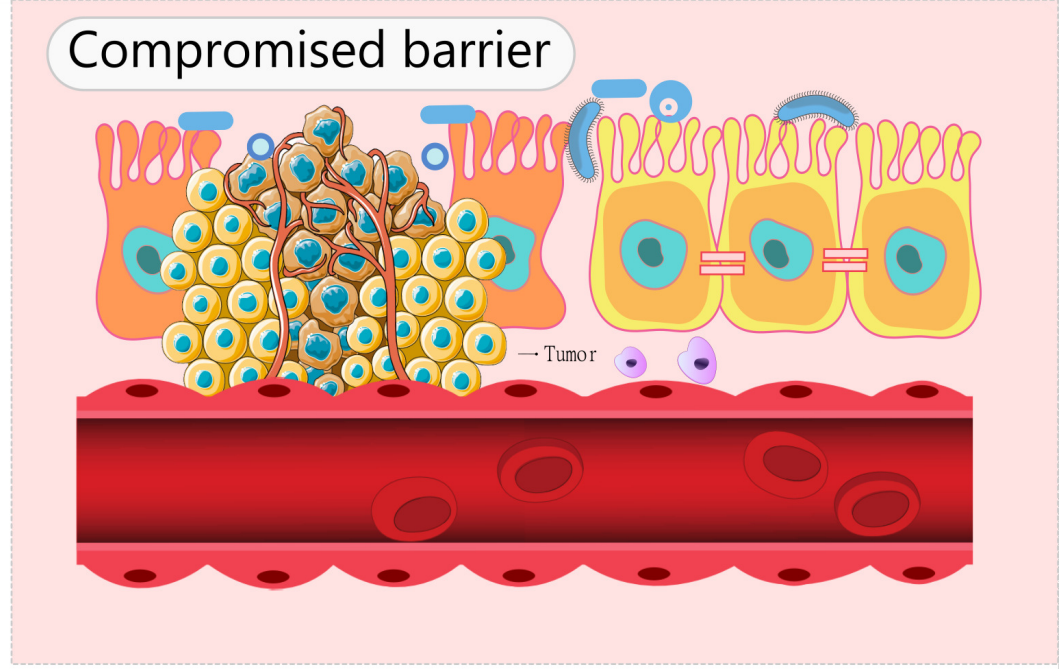
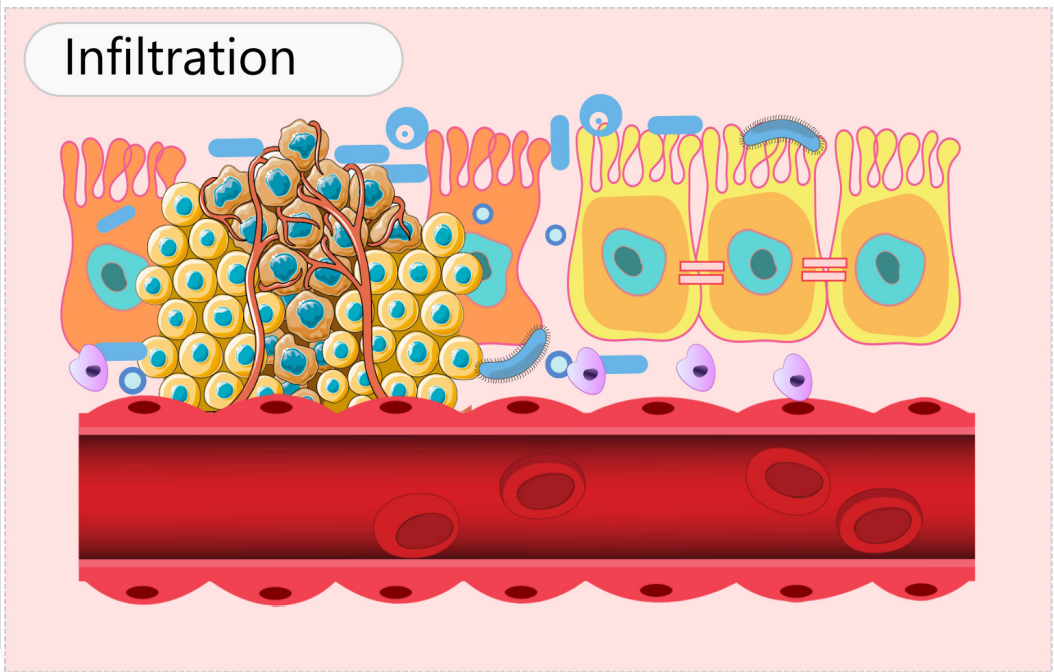
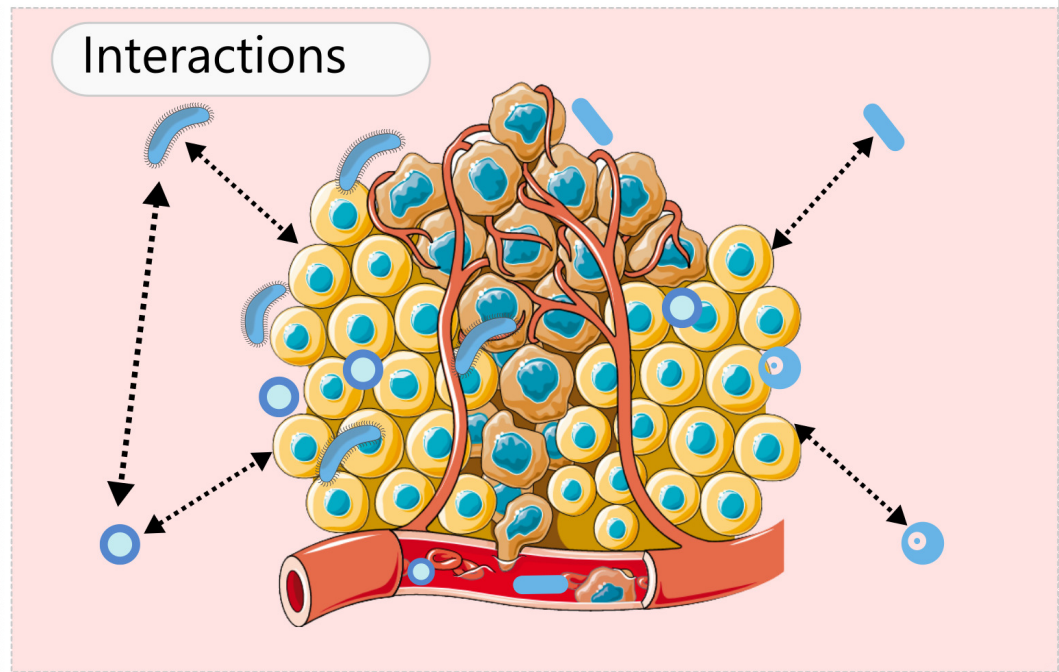

Intact barrier
Lumen
Microbes
Epithelial layer
Immune cell e.g. macrophage
Blood Vessel
Vascular Endothelium


Compromised barrier
Tumor


Infiltration


Interactions

NUTRIENT STRESS

Hyperanabolic tumor cells **create a nutrient deficit** in their microenvironment

OXIDATIVE STRESS

Aberrant growth contributes to **elevated levels of reactive oxygen species** (ROS)

PHYSICAL & OSMOTIC STRESS

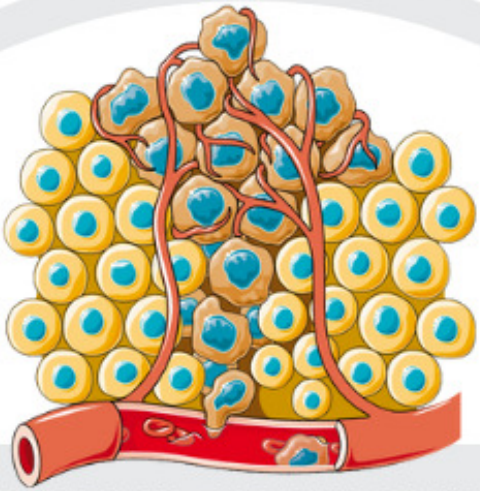
Heightened immune cell infiltration, dynamicity, NETs and ion efflux prevent colonization and create osmotic imbalance

ACID STRESS

Carbonic anhydrase, proton release and other factors mediated **hyperacidic surface of tumor** helps in metastasis/ invasion

CYTOTOXIC & DNA DAMAGE STRESS

Tumor represents an environment **replete with cytotoxic drugs, overexpressed nucleases**



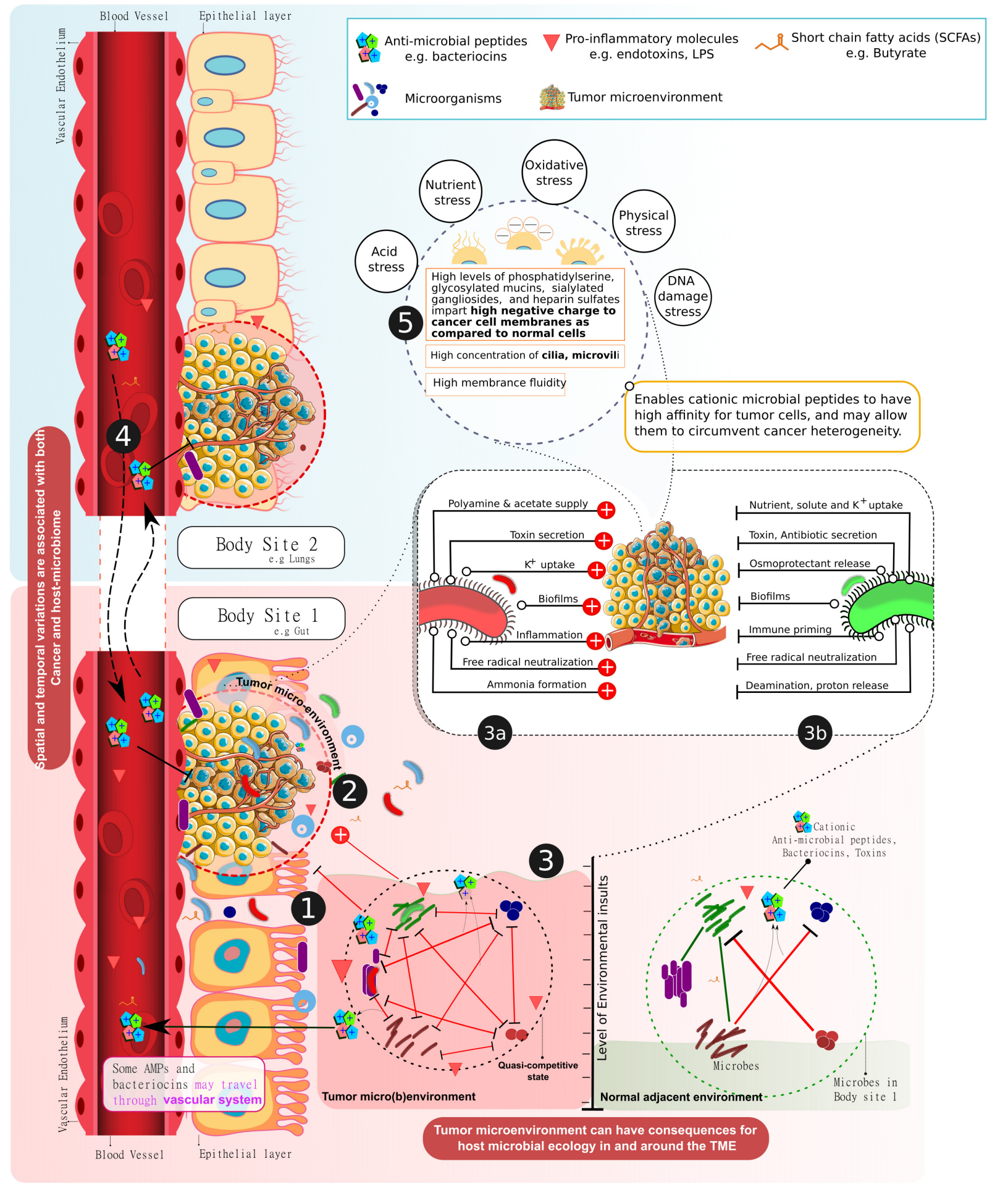
CHARACTERIZING STRESSES IN THE TUMOR MICRO(B)ENVIRONMENT

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).

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	59,63–65
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	20,66
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	15,28,67

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	25,26,31,68,69
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	50,70,71
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	41,53,72–75

** 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


Anti-microbial peptides
e.g. bacteriocins
Pro-inflammatory molecules
e.g. endotoxins, LPS
Short chain fatty acids (SCFAs)
e.g. Butyrate
Microorganisms
Tumor microenvironment
Blood Vessel
Epithelial layer
Vascular Endothelium
Nutrient stress
Oxidative stress
Physical stress
Acid stress
DNA damage stress
5
High levels of phosphatidylserine, glycosylated mucins, sialylated gangliosides, and heparin sulfates impart high negative charge to cancer cell membranes as compared to normal cells
High concentration of cilia, microvili
High membrance fluidity
Enables cationic microbial peptides to have high affinity for tumor cells, and may allow them to circumvent cancer heterogeneity.
4
Body Site 2
e.g Lungs
Body Site 1
e.g Gut
Spatial and temporal variations are associated with both Cancer and host-microbiome
Polyamine & acetate supply
Toxin secretion
K+ uptake
Biofilms
Inflammation
Free radical neutralization
Ammonia formation
Nutrient, solute and K+ uptake
Toxin, Antibiotic secretion
Osmoprotectant release
Biofilms
Immune priming
Free radical neutralization
Deamination, proton release
3a
3b
Tumor micro-environment
2
1
3
Cationic Anti-microbial peptides, Bacteriocins, Toxins
Level of Environmental insults
Quasi-competitive state
Microbes
Tumor micro(b)environment
Normal adjacent environment
Microbes in Body site 1
Some AMPs and bacteriocins may travel through vascular system
Vascular Endothelium
Blood Vessel
Epithelial layer
Tumor microenvironment can have consequences for host microbial ecology in and around the TME