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Implications for the modulation of angiogenesis

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Abstract
While there has been much recent work addressing the association of the mammalian gut microbiome with pro-angiogenesis in the small bowel, speculation involving putative/discrete tumor promoting angiogenins seems notably immature, even absent. There is certainly abundant discussion involving the cascade of pro-inflammatory cytokines, chronic inflammation, cell growth factors, toxic bacterial metabolites, and their interaction with components of the immune system, but there seems a paucity of research on induction of endothelial proliferation and the potential clinical use of natural or synthetic angiogenic inhibitors. While Bevacizumab has been approved as an angiogenesis targeted therapy in colorectal lung, and renal cell cancer, and in glioblastoma (in conjunction with other therapies), its clinical efficacy to date appears dubious. We view this observation as further rationale for future bold investigation into the isolation of specific *bacterial* angiogenins and readily synthesized antibodies directed against them.

Keywords: Angiogenesis; microbiome; tumor; monoclonal antibodies

Introduction
The initiation and regulation of angiogenesis or the so-called "angiogenic-switch" underlying tumor recruitment of new blood supply has been a highly complex and provocative issue for cancer biology [1].
There have been a number of intriguing studies in past years that have suggested that the expression of angiogenin (Ang) may be modulated by nonpathogenic endogenous bacteria [2-4]. In subsequent investigation, Li *et al* [5] reported the supernatant of *Lactobacillus acidophilus* increased blood vessel formation in an embryonic cell model.

Discussion
The role of angiogenesis via the inflammatory process has been a topic of considerable investigation. Many angiogenic mediators, including VEGF (Vascular Endothelial Growth Factor), platelet-derived growth factor (PDGF), fibroblast growth factor-2 (FGF-2), epidermal growth factor (EGF), insulin-like growth factor (IGF), hepatocyte growth factor (HGF), transforming growth factor beta (TGF-beta), tumor necrosis factor alpha (TNF-alpha), interleukin-1 (IL-1), IL-6, IL-8, IL-13, IL-15, IL-18, angiogenin, platelet activating factor (PAF), angiopoietin, soluble adhesion molecules, endothelial mediator (endoglin) may all play roles in inflammatory processes including bronchial asthma [6], rheumatoid arthritis, [7] and amyotrophic lateral sclerosis [8]. JA Joyce & JW Pollard [9] suggested that areas of hypoxia in tumors drive angiogenesis via inflammatory signals, that in turn activate NF-kB, STAT3, CCL2, CXCL-12 or TAM. M. Kanther, X Sun, M Muhlbauer *et al*. [10] demonstrated that bacterial stimulation/commensal microbial colonization induced NF-kB in zebrafish cells. This finding underscores the potential contribution of the microbiome to signaling and regulatory mechanisms that may have a role in angio/tumorigenesis.
VEGF receptor tyrosine kinases alone constitute a portion of the multiple hit hypothesis of tumorigenesis, through direct pro-angiogenic activity and by inhibiting immunity through multiple mechanisms. In their comprehensive review of the intertwined regulation of angiogenesis and immunity, Rivera and Bergers [11] discussed the bypass mechanisms tumors invoke even when aggressively treated with VEGF-trapping monoclonal antibodies, which may explain the short-lived and clinically ineffectual responses to Folkman's (1996) VEGF blockade with Avastin. Bypass mechanisms may be driven by the initial hypoxia secondary to angiogenic inhibition, which likely induces gene expression that actually drives angiogenic proliferation, and inhibits adhesion of T-cell populations to luminal surfaces of capillaries, thus blocking their extravasation into the tumor. Dendritic cell maturation may also be suppressed, thus immune suppression and pro-angiogenic activity are respectively amplified [12, 13].
It may be that an apparent pathologic imbalance between the array of genetic and metabolic mediators and angiogenic inhibitors, such as endostatin, thrombospondin-1 and -2 constitute an angiogenic disequilibrium that might in turn be normalized through the introduction of monoclonal antibodies derived from what appear to be potent soluble angiogenesis factors derived from selected strains of probiotic or other commensal bacteria.
Structurally, angiogenins are likely to be tyrosine kinases that function as phosphotransferases, translocating phosphate groups from ATP, for example, to specific substrates which in turn result in functional changes to some key target protein [14]. Tumor angiogenic kinases are either receptor kinases that transduce signals into a cell, or cytoplasmic, in support of cellular communication. Selective targeting of tumor derived/ sui generis kinases by anti-angiogenic tyrosine kinase inhibitors have been used clinically in the form of sunitinib, sorafenib, and pazopanib; while these have been approved or

are in clinical trials for adjunctive treatment of patients with advanced cancer, efficacy appears to be clinically dubious. Recent bacterial phosphoproteomic studies have confirmed that the protein phosphorylation in bacteria that was reported widely in the late 1970s now support the suggestion that this process serves to declare its prominent role in bacterial environmental signaling via conformational changes in the extracellular domains of the BY-kinases [15].

Tyrosine phosphorylation is today recognized as a key regulatory device of bacterial physiology, linked to exopolysaccharide production, virulence, stress response and DNA metabolism. However, bacteria appear to have developed tyrosine kinases that share no resemblance with their eukaryotic counterparts and are unique in exploiting the ATP/GTP-binding Walker motif to catalyze auto-phosphorylation and substrate phosphorylation on tyrosine. These enzymes, named BY-kinases (for Bacterial tYrosine kinases), have been identified in a majority of sequenced bacterial genomes.

Given the prospective validity of the basic concept summarized here, one extremely provocative and perhaps hitherto under-researched question is whether the baseline commensal populations of the microbiome (which is exquisitely sensitive to perturbations from dietary and drug variables) or whether dietary bacteria in the form of bacterial colonies resident in consumed foods, i.e., cultured dairy products, and yeast containing foods and beverages support angiogenesis more strongly.

Using a Zebrafish-P. aeruginosa model, Vasil *et al*. [16] posited that bacteria may produce potent *anti*-angiogenins such as phospholipase C exotoxins. Obviously, trading septicemia or host vascular lesions for anti-tumor effects may not be desirable.

The potential physiological significance of modulating angiogenesis through common lactic acid bacteria, for example, is unclear, still largely unstudied, and seems to demand investigation. The clinical implications of the microbiome may well be enormous. One apparently understudied aspect may involve the downregulation of tumor vascularization on epithelial and other histologic surfaces (including breast, bone, and brain tissues via an efficacious, non-chemotherapeutic intervention that may avoid classical sequelae and reduce health care costs.

Initial steps for proposed future investigation

1. Establish the methodology and confirm the results presented by Stappenbeck *et al*. [4] using gnotobiotic mice to assess intestinal angiogenesis modulated by *B. thetaiotaomicron*.
2. Using (1) as an animal model system, assess the angiogenic potential of a) selected probiotic strains, such as *L. GG* and *B. lactis*, b) the fermentation supernatant of the identified strains, and c) the combination of microbe and fermentation supernatant.
3. Using results from (2), assess the angiogenesis modulation in other tissues, such as colon, liver, lung, bone marrow and skin.
4. Using results, from (3) assess the expression of angiogenic factors, such as Ang 1, and modulation of antiangiogenic mediators, including angiostatin and endostatin, and particularly VEGF which appears to represent a critical rate-limiting step in physiological angiogenesis.
5. Using results from (3), assess the tumorigenesis modulation in the same tissues.

Potential applications

We further propose research into:

1. A method for preparing antiangiogenic substances from biologically pure cultures of non-pathogenic strains of *Lactobacillus, Staphylococcus, and Bifidobacterium* having the ability of inhibiting or reducing tumorigenesis in intestinal and dermatological tissues.
2. A method for preparing monoclonal antibodies in response to claim 1 to prevent tumorigenesis in epithelial cells of selected tissues and composed of identified angiogenic receptors, including, but not limited to vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), fibroblast growth factor-2 (FGF-2), epidermal growth factor (EGF), insulin-like growth factor (IGF), hepatocyte growth factor (HGF), transforming growth factor beta (TGF-beta), tumor necrosis factor alpha (TNF-alpha), interleukin-1 (IL-1), IL-6, IL-8, IL-13, IL-15, IL-18, angiogenin, platelet activating factor (PAF), angiopoietin, soluble adhesion molecules, and endothelial mediator (endoglin).
3. A method for treatment of carcinomas associated with neovascularization and mediated through the growth factors in claim 2.
4. While there has been much recent literature addressing the association of the mammalian gut microbiome with pro-angiogenesis in the small bowel (see the comprehensive review by Abdulamir AS *et al*. [17]), speculation involving putative/discrete angiogenins seems notably immature and is often conspicuous by its absence from the literature. There is certainly abundant speculation about the cascade of pro-inflammatory cytokines, chronic inflammation, cell growth factors, and toxic bacterial metabolites. Abdulamir [170] cites many studies associating S. bovis/ gallolyticus with proliferative or neoplastic lesions, but there seems a paucity of research on induction of endothelial proliferation and the use of natural or synthetic angiogenic inhibitors. While Avastin has been approved as an angiogenesis targeted therapy in colorectal cancer (in conjunction with established chemotherapy), its clinical efficacy to date appears dubious. We view this observation as further rationale for bold investigation into the isolation of specific *bacterial* angiogenins and readily synthesized antibodies directed against them.

Conclusion

The slowly emerging science under discussion here is intended to stimulate more energetic research into assessing the potential angiogenesis activity of commonly occurring bacterial strains (of the microbiome) and ultimately to synthesize anti-angiogenesis factor(s) from these strains, their extracellular and/or soluble components, or their fermentation supernatants. The ultimate aim of suggested investigation is to characterize and demonstrate clinically significant anti-angiogenesis properties of synthesized monoclonal antibodies against the putative angiogenesis factors using selected tumor cell lines in vitro, and ultimately in vivo among animal models with significant tumor burden.

More specifically, our suggestion is that selected strains of lactobacillus, streptococcus and bifidobacteria and their

respective fermentation supernatants may have properties that modulate tumor vascularization through multiple mechanisms. It is further hypothesized that relatively simple molecular biology may yield anti-angiogenesis (antibody) factors that may meaningfully inhibit vascularization of neoplastic malignant lesions.

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