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Pre- and post-operative antibiotics in conjunction with cytoreductive surgery and heated intraperitoneal chemotherapy (HIPEC) should be considered for pseudomyxoma peritonei (PMP) treatment

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ABSTRACT

Pseudomyxoma peritonei (PMP) is a subtype of peritoneal carcinomatosis that is traditionally treated by cytoreductive surgery (CRS) followed by hyperthermic intraperitoneal chemotherapy (HIPEC). A growing body of evidence suggests that microbes are associated with various tumor types and have been found in organs and cavities that were once considered sterile. Prior and ongoing research from our consortium of PMP researchers strongly suggests that bacteria are associated with PMP tumors. While the significance of this association is unclear, in our opinion, further research is warranted to understand whether these bacteria contribute to the development, maintenance and/or progression of PMP. Elucidation of a possible causal role for bacteria in PMP could suggest a benefit for supplementation of antibiotics to current treatment protocols.

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Introduction

Pseudomyxoma peritonei (PMP) is a subtype of peritoneal carcinomatosis characterized by multifocal peritoneal implants of mucin-secreting cancerous epithelial cells surrounded by gelatinous ascites [1]. PMP most often originates from a perforated appendiceal neoplasm, but overt signs of peritonitis are not seen. However, hypersecretion of MUC2, a member of the mucin protein family, is a consistent hallmark of the disease, and MUC2 expression is known to be modulated by a number of bacterial pathogens [2–4]. PMP treatment includes resection of the tumor and peritoneal implants by cytoreductive surgery (CRS) plus hyperthermic intraperitoneal chemotherapy (HIPEC) with mitomycin-C or melphalan [5]. PMP is closely related to more common mucinous adenocarcinomas and gastrointestinal cancers.

PMP can take on a protracted course in the low-grade histopathology, Low-Grade Mucinous Carcinoma Peritonei (LGMCP), or can rapidly evolve as a more aggressive tumor, High-Grade Mucinous Carcinoma Peritonei (HGMCP). Low-grade disease has the potential to progress into high-grade disease. Despite aggressive treatments, PMP patients frequently relapse, with median progression-free

Abbreviations: Pseudomyxoma peritonei, PMP; cytoreductive surgery, CRS; hyperthermic intraperitoneal chemotherapy, HIPEC; low-grade mucinous carcinoma peritonei, LGMCP; high-grade mucinous carcinoma peritonei, HGMCP.

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survival of 8.2 years [6]. While survival depends on tumor histopathology and stage of disease, many patients with otherwise low-grade neoplasms succumb to recurrent mucoid ascites that can cause mechanical compression of abdominal and thoracic organs and the corresponding vascular compartments. Survival is significantly better if lymph nodes are negative (5-year survival 76% and 11% for lymph node-negative and -positive patients, respectively; $p < 0.001$) [7]. Other variables affecting survival are quality of cytoreduction and disease burden [8].

While *Helicobacter pylori* is known to be associated with the development of gastric cancer [9], increasing evidence suggests that other bacteria may also be associated with other oncological histopathologies [10–13]. With the exception of *H. pylori*, little is currently understood about the mechanisms by which these microbes may contribute to the etiology of cancer. A greater understanding of this topic is certain to provide novel avenues for future cancer therapeutics/treatments.

Results and discussion

Our consortium of PMP researchers, composed of surgeons and nurses with expertise in PMP, as well as basic scientists with expertise in *H. pylori*, cancer and the human microbiome, are working together to better understand this understudied life-threatening disease. We previously found that *H. pylori* and other bacteria are present in PMP tumors (Fig. 1A) and that bacterial densities in HGMCP tumors are higher than in LGMCP tumors [14]. We also found that the percentage of CagA-positive *H. pylori* is higher in HGMCP than in LGMCP (60% vs. 9%). Based on those results, we initiated a pilot trial in 2007 to examine the safety and efficacy of *H. pylori* eradication in PMP patients. Enrolled subjects were given lansoprazole, amoxicillin, and clarithromycin for 14 days, three weeks prior to CRS/HIPEC and a second course 2–3 months post-operatively. We hypothesized that if bacteria were involved with disease progression, antibiotics could help reduce the bacterial load and positively impact morbidity and mortality. Specifically, pre-operative antibiotics could help eliminate any additional bacterial translocation into the peritoneum, while post-operative antibiotics could help eliminate bacteria associated with PMP tissue that was not eradicated by the HIPEC procedure. Outcomes were compared to control patients who did not receive *H. pylori* eradication therapy. We compared β-catenin localization in tumors harvested from treated subjects and controls [15], studied the tumor microbiome [16], and cultured bacteria directly from tumors and mucin [17].

In normal polarized gut epithelial cells, β-catenin enables key interactions between cytoskeletal proteins and cell-to-cell junctional proteins (e.g. E-cadherin) and allows cells to tightly bind [18]. These interactions are crucial for maintenance of epithelial cell polarity, regulation of cell growth, and cell-to-cell focal adhesion. During carcinogenesis, β-catenin relocates into the cytoplasm and nucleus. Loss of membrane β-catenin leads to cell separation and migration, movement of abnormal neoplastic cells to the stroma, entry into blood vessels, and metastatic spread to different tissues through the bloodstream [19]. Furthermore, following nuclear translocation, β-catenin binds to transcriptional regulators and stimulates expression of various genes, including genes required for cell proliferation [20]. LGMCP and HGMCP sample analysis showed that nuclear β-catenin was common in both disease subtypes (Fig. 2 [15]). Additionally, in HGMCP antibiotics patients, nuclear β-catenin staining was significantly decreased compared to untreated patients (Fig. 1B); a decrease was also seen with LGMCP samples, but was not statistically significant [15]. In keeping with the idea that antibiotic treatment enabled β-catenin to return to its normal structural role in the cell, the decrease in nuclear staining for both LGMCP and HGMCP was accompanied with a significant increase in membrane localized β-catenin. These results suggest antibiotic treatment alters β-catenin localization and may offer potential protection against cellular detachment, invasion, and metastasis.

We analyzed the microbiota found in tumors and mucin from 11 patients (LGMCP $n = 4$, HGMCP $n = 7$) (Fig. 1C [16]). Sequencing analysis of the V6 hypervariable region of the 16S gene was used to profile tumor associated bacterial communities. Bacterial families found in all samples include: *Comamonadaceae, Propionibacteriaceae, Enterobacteriaceae, Methylobacteriaceae*, *Chitinophagaceae,* and *Helicobacteraceae*. Other families that dominate the colonic mucosa, such as *Lachnospiraceae, Ruminococcaceae,* and *Bacteroidaceae* [21,22] are scarcely present in the PMP microbiome. This may suggest selection for particular microbes that can resist host defenses and thrive in a mucinous environment. To our knowledge, we are the only group that has successfully cultured bacteria from

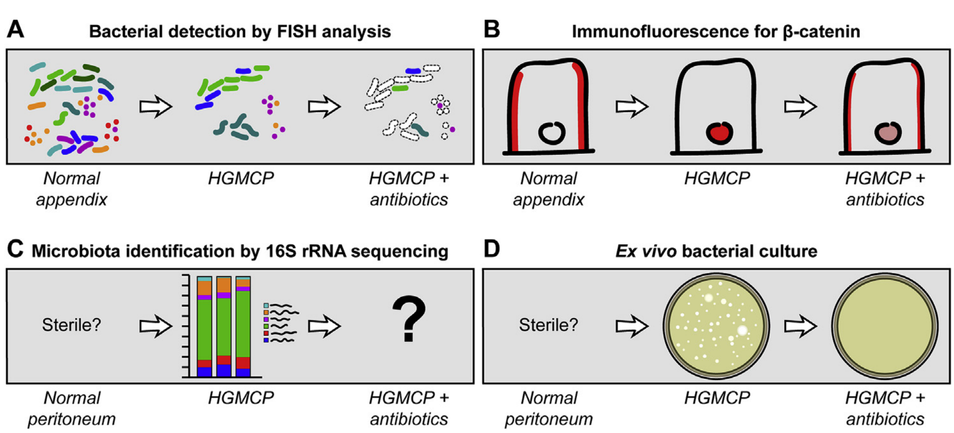

Fig. 1. Evidence from our consortium of PMP researchers shows that bacteria are present in PMP and can be altered by antibiotic treatment. **(A)** The normal appendix contains numerous bacteria. In PMP samples, *H. pylori* as well as typed and nonculturable bacteria (TNCB) have been detected by *in situ* hybridization (ISH) and fluorescence *in situ* hybridization (FISH). Antibiotic treatment decreased the number of bacteria found in PMP samples. **(B)** β-catenin in normal appendiceal samples is localized to the boundary between adjacent cells. Conversely, in PMP tissue β-catenin is localized to the nucleus. Antibiotic treatment of PMP patients resulted in relocalization of β-catenin from the nucleus to the plasma membrane. **(C)** Though it is debated, the peritoneum is generally considered to be a sterile environment. In contrast, PMP tissue and mucin samples from within the peritoneum contain a distinct microbiota. The effect of antibiotic treatment on the PMP microbiota remains unknown, but is under active investigation. **(D)** Complimentary to the microbiota studies described in (C), bacteria have been cultured *ex vivo* directly from PMP patient samples. Despite comparable efforts, no bacteria have yet been cultured from samples from antibiotic-treated subjects; this failure is consistent with the decrease in bacteria detected by FISH (A).

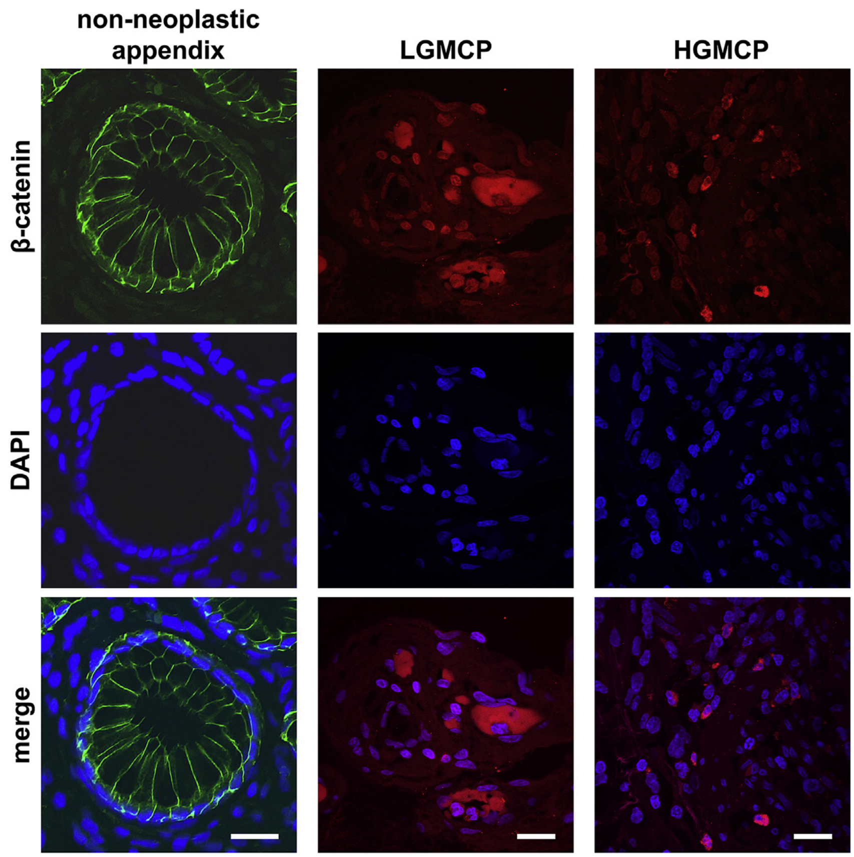

Fig. 2. β-catenin localization in non-neoplastic appendix and in pseudomyxoma peritonei samples. Formalin-fixed tissue samples from a non-neoplastic appendix control patient (left), a LGMCP patient (center), and a HGMCP patient (right) were sectioned and stained with an anti-β-catenin antibody (green or red) and DAPI (blue). The control sample shows strong β-catenin staining at the cell boundaries. In contrast, the β-catenin signal in both LGMCP and HGMCP patient samples is localized to the nucleus; little signal remains at the cell boundary. All scale bars are 20 μm.

PMP tumors. We have cultured 21 strains, most of which are highly represented in the tumor microbiome (Fig. 1D), including *Propionibacterium spp., Corynebacterium spp., Streptococcus spp.,* and *Amycolatopsis*. Some of these species, such as *Cutibacterium acnes* (formerly *Propionibacteria acnes*) and *Streptococcus,* are suspected to contribute to development of other cancers [10–12]. One interesting isolate, designated PMP191F, represents a new species in the *Chitinophagaceae* family [17]. This organism binds MUC2 and can use mucin as a carbon source [16]. These properties suggest that PMP191F may be highly adapted to the mucosal environment.

In 2013, initial survival data on 17 antibiotic subjects was reported with our microbiota study [16]. Five years later, we can update subject survival information. We reexamined pathology reports and consent documentation for all 22 participating subjects and have updated data on 17 subjects. Of these 17, two originally classified as LGMCP were reclassified as HGMCP. Of the six LGMCP subjects, one was lost to contact, one is alive with disease and the remaining four are alive without disease. Not surprisingly [7], the HGMCP subjects showed survival differences that appear to be influenced by lymph node status. Three of the four lymph node positive HGMCP subjects died of disease within 3.3 years and one remains alive without disease with 125 months of follow-up. Of the seven lymph node negative HGMCP subjects, one died of other causes, two died of disease, and four are alive without disease.

While we recognize the small study population, we feel that the survival profile is encouraging when compared to published PMP survival statistics [6]. Additionally, the complex microbiota profile [16], the ability to culture bacteria from PMP tumors [17], and the molecular studies that indicate that tumor markers may be altered with *H. pylori* therapy [15] strongly suggest that bacteria may play a role in this disease (Fig. 1). A second antibiotic protocol was initiated in 2016 (NCT02387203) as a means to increase the number of antibiotic treated patients. A larger cohort will potentially allow for a more definite conclusion about the benefit of antibiotic treatment.

While our new antibiotic study is still underway, it is interesting to think about the potential role that bacteria could be playing in PMP. For example, are bacteria driving the cancer development/progression, or are bacteria simple bystanders that are taking advantage of the tumor environment as a niche for growth and survival? These types of questions are also starting to be addressed in other areas of oncology; colorectal cancer is a prime example [13]. For PMP, the presence of *Helicobacter* and other bacteria known or thought to contribute to cancer suggests that the bacteria may be an etiological component of the cancer. Even if this is not the case, it seems almost certain that bacterial components are driving increased expression of MUC2 [2–4], which could ultimately increase patient morbidity and mortality. Furthermore, recent studies have indicated that bacteria can contribute to chemotherapy resistance [23]. Thus, in any of these models, elimination of bacteria could improve prognosis by reducing the amount of peritoneal mucin, and/or making the hyperthermic chemotherapy perfusion more effective, and/or altering the cancer development and progression.

Summary and outlook

Studies suggest that bacteria are found to be associated with several types of cancer. With the exception of *H. pylori* and gastric cancer, whether this is a correlative or causal relationship is currently not well understood. Although the sample size in our initial trial was small, the available data suggest improved survival with CRS/HIPEC and antibiotic treatment. This could suggest that bacteria promote or exacerbate PMP disease and ultimately affect patient mortality. Therefore, in our opinion the role of bacteria in this disease needs to be further explored. Furthermore, we strongly believe that elimination of bacteria through the use of antibiotics should be considered as an addition to current treatments for PMP.

Conflict of interest

The authors declare no conflict of interest.

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